Part I Full Scale Onboard Test

2 Tonsina

2.1 Full-Scale Test Reports

2.2 Study and Evaluation for Chemistry, Biology and Toxicity

2.1 Full-Scale Test Reports

Full-Scale Ozone Ballast Water Treatment for Removal of Marine Invasive Species

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1 EXECUTIVE SUMMARY

1.1 Background

The discharge of organisms found in the ballast water of oil tankers and other cargo freighters may be a major threat to public health and the environment around the world. These organisms may cause substantial economic injury in countries in whose water they are discharged. Many of these organisms are not native or established in coastal regions (including ports) where they are discharged with ballast water, and thus are collectively referred to as nonindigenous species (NIS) or invasive species. NIS can substantially disrupt the structure and function of coastal marine ecosystems. The U.S. Coast Guard also estimates that NIS introductions cause approximately $6 billion in economic damage in the United States annually. For example, the U.S. government estimates that over the past 10 years it has cost nearly $4 billion to repair damage caused by the non-indigenous zebra mussel alone, impacting shorelines, water treatment, and power generating stations in and around the Laurentian Great Lakes.

Although many transfer mechanisms (or vectors) have contributed historically to the invasion of coastal habitats by aquatic NIS, shipping has been the vector responsible for most known invasions. The rate of new invasions appears to be increasing over time, and many of these invasions are attributed to the transfer and discharge ships' ballast water. In short, ballast water is contributing strongly to the overall increase in newly detected invasions in coastal marine ecosystems.

Ballast water exchange is currently the only management strategy available for ships to reduce the quantities of non-indigenous coastal organisms in ballast water. Ballast water exchange, or mid-ocean exchange, occurs when ships replace coastal water in their ballast tanks with open ocean water to reduce the abundance of coastal NIS. It is a management strategy that many ships can implement immediately, and which does not require retrofitting or development of new technology.

Ballast water exchange (BWE) has some significant limitations and is viewed generally as a stopgap measure to reduce the risk of invasions. First, it is not always possible to safely conduct an exchange, because of risks to the structure and safety of vessels (especially in heavy seas). Second, even when performed, BWE still leaves a residue of coastal organisms. Third, for many voyages of short duration (e.g., coastwise transits limited to a hours or a few days), sufficient time may not exist to complete ballast water exchange, and the distance from shore may be insufficient to be entirely effective (as described above).

Therefore, efforts are now underway to develop and implement technological alternatives to ballast water exchange. Although many treatment possibilities are being explored, their evaluation is at an early stage and no alternative treatments have yet been approved by state, regional, or federal regulatory authorities. At the present time, the U.S. Coast Guard (as directed by the National Invasive Species Act of 1996) and some states require that alternative treatments be, at a minimum, as effective as BWE. However, no specific guidelines or minimum standards of efficacy currently exist to assess the performance of these alternative treatments.

1.2 Testing the Effectiveness of Ozone as a Potential Treatment Technology

In 1998, British Petroleum Alaska and Nutech O3, Inc. (hereafter referred to as BP and Nutech) undertook the development and testing of ozone gas as a potentially effective alternative method of decontaminating ballast water that contains NIS. A full-scale prototype ozonation system was installed in September 2000 and tested on board the BP-affiliate ship the *S/T Tonsina* (Alaska Tanker Company), a 869-foot, double-hull oil tanker with 12 segregated ballast water tanks, with a total capacity of approximately 11,000,000 gallons (41,365,000 L).

BP and Nutech subsequently partnered with several academic and industrial research institutions to design and implement a rigorous, independent analysis of the ozone system's ability to remove non-indigenous or invasive species from marine ballast water. The study described in this report represents the first of several experimental phases planned to provide a full evaluation of the efficacy of the prototype Nutech ozone system aboard the *S/T Tonsina*. The primary goal of this present (Phase 1) study was to conduct a field-scale test of the operation and efficacy of this ballast water treatment system for removal of a wide range of coastal marine organisms.

The specific objectives of the present study were to:

1) Determine the efficacy of a full-scale ozone system to remove coastal organisms compared to ballast water exchange.

2) Assess the possible environmental risks of discharging ozone-treated ballast water by measuring chemical constituents of the water over time and using whole effluent toxicity testing to assay the latent toxicity of the ballast water at the time of discharge.

3) Obtain operational experience with the prototype ozone system in order to implement further system improvements.

In short, this first phase represents a "proof of concept" for the Nutech ozone treatment system, providing key data needed to address each of the three primary objectives. It is important to recognize the current data, in Phase 1, are limited to a few trials from one port system.

1.3 Experimental Design

This study is the first of several phases, and measured the effects of ozone treatment and ballast water exchange, replicated on multiple dates with ballast water originating from Puget Sound. The experiments were designed to compare changes in treatment tanks over time to those observed in untreated control tanks. Treatment tanks (designated for ozone or ballast water exchange) were filled from the same source as untreated control tanks and all tanks were sampled at fixed time points throughout the same experiment.

Three ozone experiments and two ballast water exchange experiments were conducted. Including a third tank as a control, ballast tanks were filled at the same time and location to obtain a direct comparison between the efficacy of exchange and ozonation. Samples were

collected at multiple time points, including before and after treatment, from each tank using several access locations (manways or Butterworth® openings) on the deck of the ship. Treatments were as follows: No. 3 wing port (ozone treatment); No. 3 wing starboard (air-sparged control); and No. 4 port (ballast water exchange). Samples were used to measure changes in biota and water chemistry over time, as described below.

Effects of treatment on biota were measured in two ways. First, for organisms entrained in the ballast tanks, samples were collected from treatment and control tanks at least before and after treatment, and sometimes at intermediate time points, to compare changes in concentration and condition of resident organisms between treatments. This approach was used to measure effects of ozone and ballast water exchange treatments on bacteria, phytoplankton, and zooplankton. Second, for larger organisms (which are rare and more difficult to sample), a defined number of individual organisms were placed in various types of cages to measure the effect of ozone treatment. This second approach was used for fish, crabs, mysids, and amphipods. These caged organisms were placed in ozone treated and control tanks to compare mortality rates over time; a similar approach was not used in the BWE tanks, due both to the turbulence associated with this treatment and the mode of action, which was considered to be primarily achieved through removal and not mortality.

One preliminary and three full experiments were conducted over the course of one year. The preliminary test, designed to provide data for the full scale testing, provided information on the chemical reactions of ozone, including by-product formation and their effects on bacteria. Experiment 1 closely mimicked the ozone dosage that could be achieved on the *S/T Tonsina* during routine operations. During a typical 3.5-day voyage, the ozone system would apply 0.62 mg/L/hours ozone to the 2,850,000 L of each segregated ballast water tank in the vessel for a duration of five hours. This would be achieved by treating the 12 segregated ballast water tanks separately. During experiment 1, the ozone-loading rate was 0.59 mg/L/hours and lasted 5 hours. Experiment 2 achieved an ozone-loading rate of 0.86 mg/L/hours that resulted from improved operation of the ozone generator. In experiment 3, where only the vertical portions of the tanks were treated and the experiment lasted for 10 hours, an ozone-loading rate of 1.35 mg/L/hours was achieved. In Experiments 2 and 3, much larger amounts of ozone were purposely directed to the tank compartments that were sampled.

1.4 Results

1.4.1 Efficacy of Ballast Water Exchange

Ballast water exchange removed an average of 64% of the target animals measured in the first two exchange experiments (Figure 1.1). For each experiment, 5 coastal organisms were selected, on the basis of their abundance and restricted coastal distribution, to provide a quantitative measure of exchange efficacy. Figure 1.1 indicates the percent reduction observed in the ballast water exchange treatment relative to the control treatment of each of the target taxa. The data are displayed by experiment, indicating the variation observed among taxa. Despite considerable variation among taxa, the mean efficacy among taxa was similar between experiments: 59 % and 69 %.

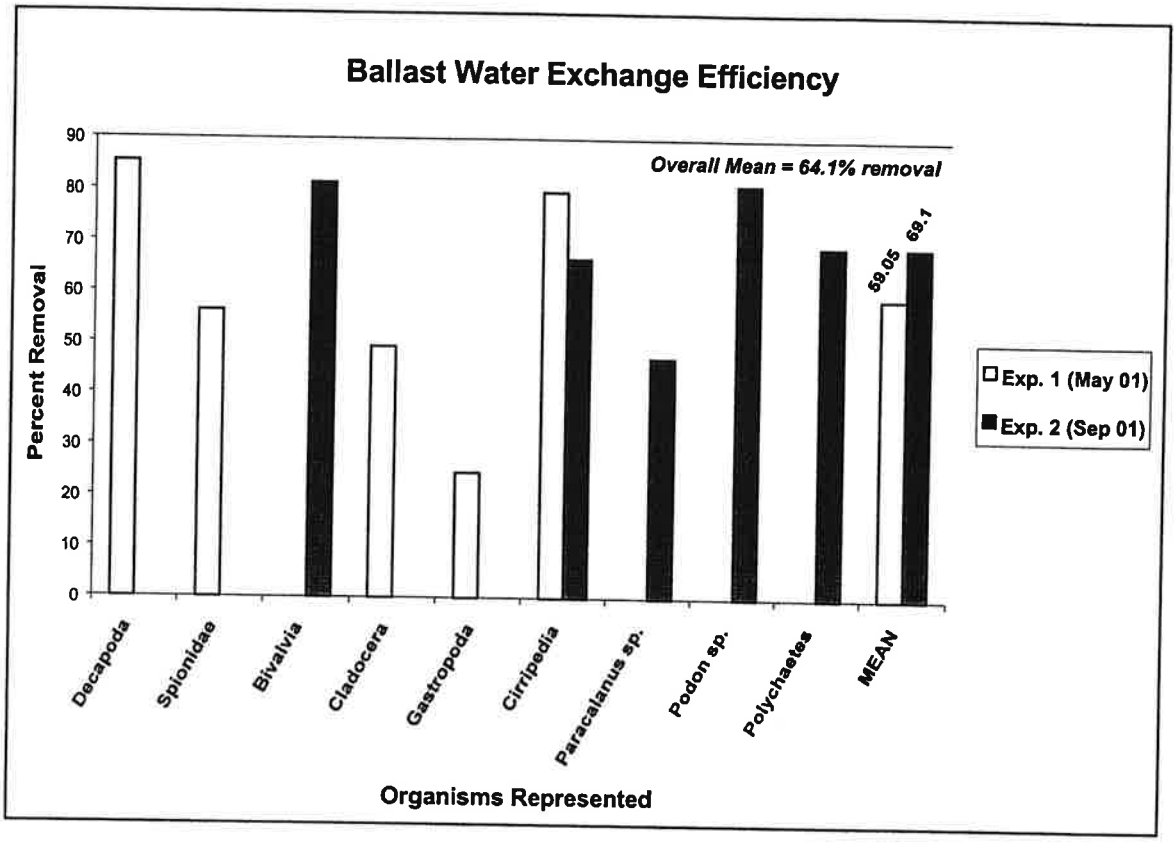

Figure 1.1. Summary of percent removal of marine organisms using ballast water exchange.

The efficacy of BWE, measured aboard the *S/T Tonsina*, was considerably lower than some proposed regulatory targets of 95 %. This level of reduction also appeared lower than that measured on other vessels, resulting perhaps from the structural complexity of the *S/T Tonsina's* ballast tanks relative to the other vessels examined to date.

The direct comparison of BWE and ozone treatment on the same vessel is critical in evaluating the ozone treatment effectiveness. Moreover, our results (1) underscore the variation the can exist within ship type, and (2) suggest the level of "kill" needed for ozone treatment to surpass ballast water exchange aboard the *S/T Tonsina* may be lower than that for other vessels.

1.4.2 Ozone Chemistry

In seawater where there is a significant concentration of bromide ion (Br^-), ozone is catalytically destroyed with a half-life of five seconds. As expected, there was no ozone observed in any of the ballast water samples we analyzed. Therefore, ozone *per se* can be considered a good oxidant for the disinfection of marine ballast water because it is not chemically persistent.

Bromate ion (BrO_3^-) was never detected at measurable levels in the treated ballast water, suggesting that the lower pH of the coastal water favored the formation of hypobromous acid (HOBr). Ozone and its residuals apparently did react with naturally occurring organic matter resulting in the formation of modest concentrations of bromoform in our experiments. The appearance of bromoform, and the fact that no bromate ions (or chloroform) were detected in any of the experiments, indicates that bromine (represented by hypobromous acid/hypobromite ions, or HOBr/OBr^-) was formed in significant quantities during the ozonation process.

Concentrations of ozone-produced oxidants (i.e., bromine) were measured in ballast water using an electrode measurement of Oxidation-Reduction Potential (ORP), and a chemical measurement for Total Residual Oxidants (TRO). Ozonation increased ORP levels up to a plateau of ca. 700-800 millivolts (mV), which is consistent with seawater disinfection targets used by commercial marine exhibit aquaria. TRO levels exceeded limits of analytical detection (4 mg/L as chlorine equivalents) in most of the experiments on board the *S/T Tonsina*. The scientific literature suggests that even 4 mg/L TRO should exceed concentrations known to be acutely toxic (e.g., 1-2 mg/L) to many marine organisms.

1.4.3 Efficacy of Ozone Treatment in Ballast Water Tanks

Figures 1.2 and 1.3 summarize the efficacy of ozone treatment for different organisms, for the different experiments, and time of ozonation at the time of sampling. Figure 1.2 summarizes the results of "killed" organisms while Figure 1.3 summarizes the total for the killed and moribund organisms. Efficacy for each organism is estimated as (a) the percent reduction in initial concentration for bacteria, microflagellates and dinoflagellates or (b) the percentage of sampled organisms that were dead or moribund for zooplankton, sheepshead minnow and mysid shrimp. The results are compared to the 64 % BWE efficacy (i.e., percent removal) as measured for zooplankton on the *S/T Tonsina* (Section 1.4.1). The percent removal for each group is shown, along with an indication (denoted by bars labeled with *) of whether percent removal of that particular organism by ozone was greater than that of mean BWE performance on this vessel.

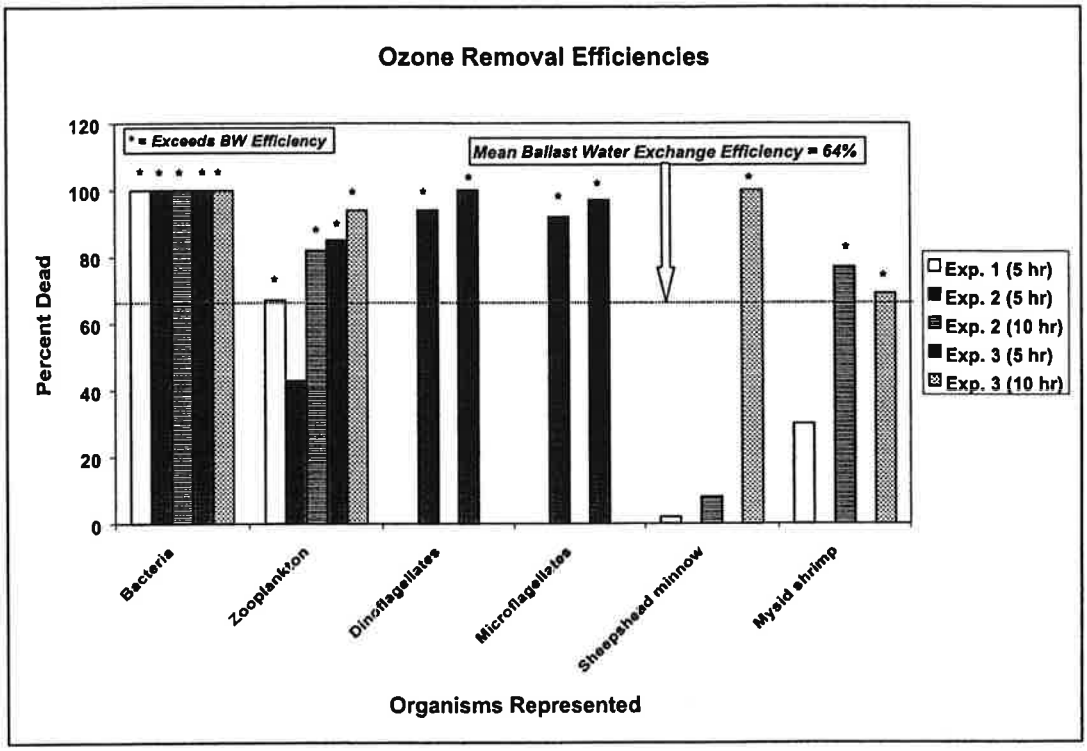

Figure 1.2. Percent mortality in ozone treatments vs. 64% mean organism removal efficiency from ballast water exchange on the *S/T Tonsina.*

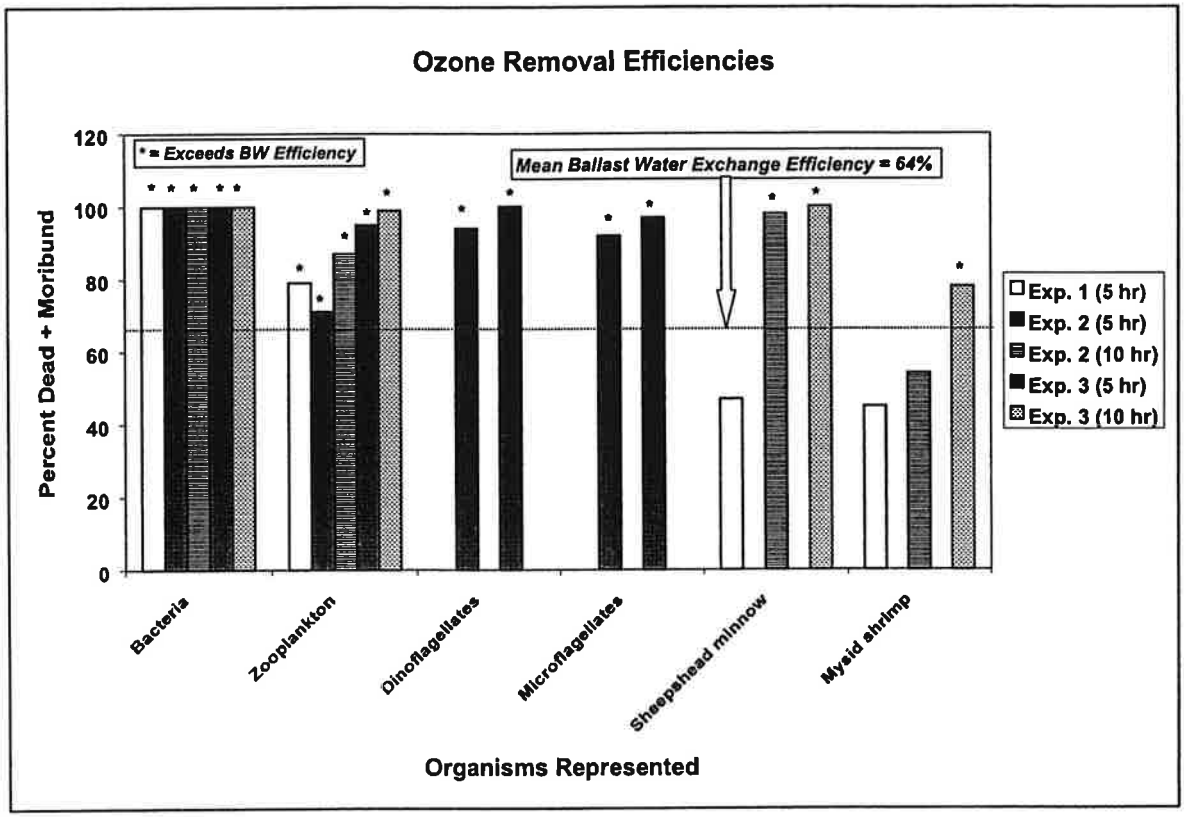

Figure 1.3. Percent dead + moribund in ozone treatments vs. 64% mean organism removal efficiency from ballast water exchange on the *S/T Tonsina*.

For the ozone treatment, the following results were observed (relative to the paired control treatment):

1. The concentration of culturable bacteria declined 99.9 %.

2. The zooplankton examined were determined to be 71-99 % dead or near death (moribund).

3. The concentration of vegetative cells for dinoflagellates and microflagellates declined 92 – 100 %. The effects of ozonation on diatoms have not yet been measured.

4. Results for larger, caged organisms were more variable. Among experiments, mortality was as follows: 2-100 % for sheepshead minnows, and 30-77% for mysid shrimp (Figures 1.2). For the sheepshead minnow and mysid shrimp, many organisms appeared moribund and may have been seriously impaired by the treatment, potentially increasing the overall effect of the ozone treatment (Figure 1.3).

5. Mortality rates for benthic organisms (e.g., amphipods and shore crabs) tended to be low. However, in contrast to the sheephead minnows and mysid shrimp, the amphipods and crabs did not exhibit noticeable signs of stress that could result in long-term mortality.

6. The efficacy of ozone treatment generally surpassed that for BWE for bacteria, zooplankton, and phytoplankton.

7. For the larger organisms, it is presently not possible to compare the results of ozone treatment to BWE. We presume exchange would reduce the concentration of these organisms, but it remains difficult to obtain such data for large, mobile organisms.

8. Studies using known numbers of caged organisms suspended in ballast water tanks generally confirmed the level of ozone efficacy, as well as the relative sensitivity of various marine species.

1.4.4 Laboratory Toxicity Tests

The effect of various ozone exposure concentrations and durations on marine organisms was also studied using controlled laboratory experiments. Median lethal concentrations (i.e., [1]LC50) for all but one species exposed to ozonated artificial seawater in the laboratory ranged from 698 - 768 mV ORP, and from 1.29 - 2.93 mg/L TRO. 50% mortality was never achieved for the amphipod, *Leptocheirus plumulosus*. These data were consistent with results from the caged organism studies in which mortality (at least for mysid shrimp) also was strongly correlated to ORP measurements. Therefore, ORP measurements ranging from 700-800 mV

[1] LC50 represents the concentration of a chemical that causes 50% mortality in an acute toxicity test

appear to be associated with significant acute mortality in a variety of marine species both in the field and in the laboratory.

Furthermore, the relative sensitivity of test species exposed to ozone (as measured by ORP) was similar in both the field and lab experiments. In the caged studies, the sheepshead minnow *C. variegatus* was the most sensitive species, followed by mysids (*A. bahia*) and amphipods (*R. abronius*). In the laboratory, LC50 values for *C. variegatus* were indeed lower than *A. bahia*, suggesting that the sheepshead minnow was slightly more sensitive with respect to ORP exposure. One of the amphipod species tested in the laboratory (*L. plumulosus*) was less sensitive to ORP than either sheepshead or mysids. Thus, laboratory studies provided a realistic indication of ozone toxicity to various species.

1.4.4.1 Toxicity of Ballast Water Following Ozonation

A major concern following treatment of ballast water with any biocide is the discharge of potentially toxic chemicals to the environment. For ozonated seawater, bromine is the residual oxidant most likely to exist for any extended period of time, in concentrations potentially harmful to marine organisms. Therefore, we conducted a series of laboratory tests with ozonated seawater generated either from the main *S/T Tonsina* experiments, or using a similar laboratory-scale ozone generator. The goals of these studies were to evaluate whether ozone residuals may be toxic in seawater and whether this toxicity may persist over time. The following discussion is a summary of those tests.

1.4.4.2 Whole Effluent Toxicity Tests

As part of the regulatory process for the approval of a ballast water chemical treatment process, the treated water will likely need to be screened for potential toxicity using standard whole effluent toxicity (WET) tests. WET tests are widely conducted as part of routine monitoring of wastewater discharges regulated under the federal Clean Water Act. Results of the WET tests using ozone-treated ballast water with the mysid shrimp, *Americamysis bahia,* and the topsmelt, *Atherinops affinis*, indicated that ozonation byproducts were stable enough to cause toxicity in ballast waters 1-2 days after ozonation and at dilutions of from 30 – 80 %. However, no chemical measurements were conducted in these tests to quantify concentrations of ozone-produced oxidants.

1.4.4.3 Latent Toxicity Tests

To validate the WET test results, mysid shrimp were exposed to ozone (using 4-5 hours of ozonation) in the laboratory using experiments of similar design to the WET tests. We initiated tests with ozonated waters that were stored for 0, 24 or 48 hours and measured toxicity along with ORP and total residual oxidant (TRO) over time. As expected from the WET tests, residual oxidants did not disappear from ozonated waters held in the dark 24 or 48 hours in a sealed container at 12 °C. All organisms died when exposed to 50, 75, (diluted) or 100 % (non-diluted) water that had been ozonated and stored either 0, 24 or 48 hours. In treatments where 100 % mortality occurred by 24 hours, the ORP was greater than 720 mV, and TRO greater than 1.76 mg/L.

We also evaluated whether relatively short-term ozonation might generate sufficient oxidant (i.e., bromine) to cause acute mortality to mysid shrimp transferred to clean seawater 1-2 days following ozonation. Limited mysid mortality (30-60%) occurred within the 1.5 hours of ozone exposure in laboratory experiments where TRO concentrations exceeded 4.0 mg/L. However, 100% mortality was observed in those survivors 48 hours after transfer to clean seawater. No mortality was observed within 1.5 hours of ozonation, or at 24 hours post-exposure when TRO measurements were less than 1.0 mg/L, but 60% mortality occurred in these same treatments after 48 hours of post-exposure. Therefore, it appears that sufficient amounts of bromine oxidants built up in the ozonated water over 1.5 hours to have induced both immediate and, to an even greater extent, delayed mortality after transferring organisms to clean water (up to 48 hours later).

The presence of bromine thus may cause both immediate and delayed toxicity to marine organisms even after relatively short periods of ozonation. Preliminary experiments suggested, however, that this residual bromine may be easily removed using commonly available reducing agents such as sodium thiosulfate, and thus this could remove toxicity from ozonated ballast waters prior to discharge. Bromine also is likely to be quickly destroyed (i.e., chemically reduced) upon discharge into marine surface waters, and so may be of only limited environmental/regulatory concern for ballast water discharge. Additional study is warranted to verify this conclusion.

1.5 General Conclusions and Recommendations

Results from this (Phase I) study, using the prototype system on board the *S/T Tonsina,* suggest that ozonation can be effective at removal of many coastal organisms from full-scale ballast tanks and may compare favorably with BWE. Key conclusions of our study include:

1. Using this prototype system, 5-10 hours of ballast water ozonation resulted in a 71-99% reduction of selected marine phytoplankton, zooplankton and bacteria. The results depended upon the individual organism and the amount of ozone gas delivered to individual ballast water tanks over time.

2. Large, mobile organisms (especially benthic crabs and amphipods) appeared to be relatively resistant to ozone treatment compared to planktonic organisms.

3. Our experiments may have underestimated the efficacy of ozone treatment resulting from the possible residual toxicity of bromine over time. Some organisms appeared affected by the initial treatment and may succumb over time, however, such effects are not included in our analysis. Additional study under field conditions is warranted to test for such effects.

4. The efficacy of ozone treatment to reduce planktonic organisms was as good as that of BWE aboard the same vessel for which empty-refill exchange resulted in an average reduction of 64% for zooplankton.

5. Both field and laboratory experiments suggested that significant organism mortality can be achieved once concentrations of ozone-produced oxidants reach 1 – 3 mg/L (as chlorine equivalents), or when oxidation-reduction potential reaches levels of 700 – 800 mV. Once further validated, such toxicity thresholds could be used to help develop control targets for aiding the routine operation of ozone systems.

6. Our preliminary results suggested that bromine was the ozone-produced oxidant that was responsible for organism mortality. Furthermore, bromine may persist at toxic concentrations in ballast waters 1 - 2 days following ozonation depending on storage conditions and exposure to sunlight.

2 INTRODUCTION

2.1 The Problem

The worldwide transfer and introduction of nonindigenous species (NIS), or invasive species, by human activities is having significant and unwanted ecological, economic and human-health impacts (e.g., OTA 1993, Wilcove et al. 1997, Pimentel et al. 2000). Although most attention to date has focused on invasions in terrestrial and freshwater habitats, it is evident that NIS invasions have become a potent force of change in coastal marine ecosystems. Roughly 400 marine and estuarine NIS are known to have been established in North America alone and over 200 of these species can occur in a single estuary (Cohen and Carlton 1995, Ruiz et al. 1997, 2000a). Some of these species have become numerically or functionally dominant in invaded communities, where they have significant impacts on population, community and ecosystem-level processes (e.g., Cloern 1996, Crooks 1999, Ruiz et al. 1999, Grosholz et al. 2000).

Although many transfer mechanisms (or vectors) have contributed historically to the invasion of coastal habitats by NIS, shipping has been the vector responsible for many of the known invasions (Carlton 1979, Carlton and Geller 1993, Cohen and Carlton 1996, Hewitt et al. 1999, Ruiz et al. 2000a). Furthermore, the global movement of ballast water now appears to be the single largest transfer mechanism for marine NIS. Since the 19th century, ships have used ballast water for stability, discharging water both at ports of call and en route (Carlton 1985). Ports receive relatively large volumes of ballast water originating from source regions throughout the world. For example, the United States and Australia each receive annually over 79 million metric tons of ballast water on ships arriving from foreign ports (Kerr 1994, Carlton et al. 1995). A taxonomically diverse community of organisms is entrained and transported within ballast tanks (e.g., Carlton and Geller 1993, Smith et al. 1999, Hines and Ruiz 2000, Ruiz et al. 2000b), resulting in many successful invasions of nonindigenous species at ports throughout the world.

BWE or mid-ocean exchange is currently the only management strategy available for ships to reduce the quantities of non-indigenous coastal plankton in ballast water (National Research Council 1996). Ships practice two types of BWE that replace coastal water with oceanic water, reducing the initial concentration of coastal organisms (i.e., those that are most likely to invade a port). Flow-Through Exchange occurs when water from the open ocean is pumped continuously through a ballast tank to flush out coastal water, and Empty-Refill Exchange occurs when a tank is first emptied of coastal water and then refilled with oceanic water.

The National Invasive Species Act of 1996 (NISA) created a program in which vessels arriving from outside of the Exclusive Economic Zone (EEZ) voluntarily conduct open-ocean exchange, or use an approved alternate treatment of ballast water permitting ballast tanks to be discharged in U.S. ports. More recently, individual states (e.g., California, Maryland, Oregon, Washington and Virginia) have passed and implemented similar laws, sometimes making this management mandatory.

BWE is viewed generally as a "stop-gap" measure to reduce the risk of invasions. It is a management strategy that many ships operators can implement immediately and does not require retrofitting or development of new technology. However, ballast exchange has some significant

limitations. First, it is not always possible to safely conduct an exchange in high seas. Second, some risks to the structure and safety of vessels in "bad weather" exist and may prevent exchange. Third, the data for the efficacy of BWE are incomplete; however, in any case ballast exchange leaves a residue of coastal organisms in the ballast tank where they contaminate the exchanged water.

Efforts are now underway to develop and implement technological alternatives to BWE. Although many treatment possibilities are being explored (e.g., NRC 1996, Hallegraeff 1998, http:/www.invasions.si.edu), their evaluation is at a very early stage and no alternative treatments have been approved.

At the present time, the U.S. Coast Guard (as directed by NISA) requires that alternative treatments be at least as effective as BWE. However, there exist no specific guidelines to assess the performance of treatments. In Appendix B, we present a conceptual framework for evaluation of alternative treatments and, based on this framework, we designed and executed a study protocol to measure the efficacy of ozonation as a specific treatment system. This report presents the results of pilot studies designed to evaluate the efficacy of ozonation as an alternative system for removal of nonindigenous species from marine ballast water.

2.2 Goals and Objectives

The goal of the study conducted in autumn 2001 was to conduct a field-scale test of the operation and effectiveness of the Nutech ozone ballast water treatment system. While preliminary studies (Section 2) suggested that the process was likely to be effective, its performance with respect to higher organisms at the field scale was as yet untested. Therefore, the present study evaluated the efficacy of ballast water ozone treatment when applied to a wider range of aquatic organisms in a full-scale oil tanker installation, using ballast water collected in the Puget Sound region prior to the *S/T Tonsina's* return to Valdez, AK. Three tests were conducted: one involving 5 hours of ozonation on September 24 and two involving 10 hours of ozonation on November 2 and November 4, 2001.

The specific objectives of the study were:

- To evaluate the chemical and biological quality of ballast water in the treated *vs.* control ballast water tanks over the course of the ozonation periods. Several types of data were collected to assist in this evaluation:
    - Concentrations of ozone and its residuals, along with basic water quality characteristics;
    - The abundance and diversity of several taxa of marine biota normally entrained into ballast water tanks (e.g., bacteria, zooplankton, phytoplankton); and
    - The survival of caged marine organisms of known identity and abundance
- To estimate the reduction of selected organisms by ozone treatment as compared to similar measures for BWE.
- To evaluate the potential toxicity (via ozone and/or its by-products) of post-treatment ballast water prior to discharge using whole effluent toxicity (WET) tests.

The current study (Phase I) was intended as a proof-of-concept for ozone treatment that will be further tested in additional phases. Using grant money from the U.S. Fish and Wildlife Service and the NOAA National Sea Grant College Program, the next phases will evaluate more complex aspects of spatial complexity in ozonation effectiveness and other possible sources of variation including water quality and composition of the entrained biotic community (Section 9).

3 LITERATURE REVIEW

3.1 Efficacy of Ballast Water Exchange

The exchange of ballast water taken on board while in port and the replaced water with oceanic ballast water during a voyage is the only available method for ship owners to reduce the transfer of aquatic organisms. BWE is also the only method specifically supported by national and international regulations at this time. However, the efficacy of BWE is limited because it is not possible to completely replace all of the water, sediments and associated biota resident in ballast tanks during an exchange. Also, the stress on hulls created by BWE can make it unsafe for some ships to undertake exchanges, especially in heavy seas.

The current standard for BWE procedures promulgated by the International Maritime Organization (IMO) is 300 % exchange by volume for the flow-through method and 100 % exchange for the empty-refill method (IMO Resolution A.868(20), 1997). These standards provide a theoretical level of at least 90 % replacement of coastal water by oceanic water, but this has not been adequately validated by field studies (Hines et al, 2000). The exchange of ballast water does not necessarily imply that an equivalent exchange of organisms occurs. In fact, the efficacy of organism removed will vary considerably among different organisms depending upon their size, mobility, behavior and whether the organism is associated with the water column or the benthos (the sediment that may accumulate in the bottom of the ballast tanks).

Because of the limitations of BWE, alternative methods for treating ballast water are actively being developed and tested. In the United States, the U. S. Coast Guard encourages such development and will approve treatment methods demonstrated to be at least as effective as BWE (USCG, Federal Register: Page 17782-17792, April 10, 1998). However, the effectiveness of BWE is poorly documented and not well understood (e.g. Everett, 2001). This is due not only to the relatively few studies that have attempted to document the effectiveness of BWE, but also to the varying methodologies which have been used in those studies.

Ten such studies undertaken by various authors between 1988 and 2000 and compiled by the U.S. Coast Guard attempted to document the effectiveness of more than 100 BWEs conducted by bulk carrier and container ships (Everett, 2000, unpublished report). These studies measured ballast exchange effectiveness in terms of volumetric water replacement at between 87.8% and 99%, and/or in terms of removal of selected planktonic organisms at between 48% and 100%, with occasionally significant variation between the two types of measurement.

Another study was undertaken in 1998 and 1999 on oil tankers similar to the *S/T Tonsina*, which were engaged in trade patterns nearly identical to the *S/T Tonsina's* (transport of Alaska North Slope crude oil from Valdez, Alaska to refineries on the U.S. West Coast). In six

exchange experiments aboard these tankers, volumetric exchange of ballast water was measured, using rhodamine dye as a tracer, to compare 300% flow-through exchange in one tank to 100% empty-refill exchange in another. In these comparative experiments, both types of exchange appeared to achieve very high volumetric water replacement percentages (~99%), although the empty-refill method was able to accomplish this after one volume was exchanged rather than the two or three required by the flow-through method (Ruiz et al., unpublished data).

Although these studies are helpful in illustrating the range of effective BWEs, any generalization of these effects across ships and organisms is premature. This results both from the limited number of measurements and the diversity in methodology that limits direct comparison.

Thus, to effectively compare the efficacy of any treatment (e.g. ozone) to BWE it is necessary to measure concurrently the performance on the same vessel with the same methods. We included BWE experiments in the study design for this project using water that was taken up at the same time and place as water used in the ozone experiments so that direct comparability between the two treatment methods could be achieved.

3.2 Ozone Chemistry: A Brief Review of Fresh and Marine Waters

Ozone has been used as a disinfectant since the late 1800's. It is used widely in Europe in drinking water treatment and to a lesser extent in the U.S. (Hoigne, 1998). It is an oxidant and biocide and is unstable in water (Langlais et al., 1991).

An excellent discussion of ozone decomposition in water that does not contain bromide has appeared in a publication authored by Staehelin and Hoigne, 1985. Fundamentally, ozone decomposition is a base-promoted decomposition with a half-life of 20 seconds at pH = 9. With decreasing pH, the half-life increases by an order of magnitude for each decrease in one pH unit. Ozone decomposition is a chain reaction that involves the formation of the hydroxyl radical, ·OH. The initial reaction of ozone with OH^- results in the formation of superoxide anion radical $O_2^{-}\cdot$. The $O_2^{-}\cdot$ is in equilibrium with its protonated form $HO_2\cdot$ with a pK_a (equilibrium constant) = 4.8 (Bielski et al., 1985). It was noted that the presence of organic and some inorganic compounds promoted the decomposition of ozone. These reaction by-products are transient species and may be involved in the disinfection process, but would not persist in solution.

The biggest difference between ozone chemistry in water treatment and treating marine ballast water is the presence of bromide ion in seawater (Oemcke and van Leeuwen, 1998). Bromide ion catalytically decomposes ozone according to Figure 3.2.3 (von Gunten and Oliveras, 1998) and other studies (Salhi and von Gunten, 1999; von Gunten and Hoigne, 1992; von Gunten et al., 1996; von Gunten and Oliveras, 1997; Pinkernell et al., 2000; Pinkernell and von Gunten, 2001; von Gunten et al., 2001). Two relatively stable by-products are formed when ozone is used to treat seawater, bromate ion and bromoform. The formation of these by-products is through the oxidized bromide ion (bromine). In seawater, bromine rapidly forms hypobromous acid, which is in equilibrium with hypobromite ion. It is also possible to form monobromamine if the concentration of ammonia is sufficiently high. Monobromamine is unstable and will decompose to ammonia and bromide ion (Hofman and Andrews, 2001).

The following equations describe the chemistry shown in Figure 3.2.3. Where they are known, reaction rate constants or equilibrium constants are included (Haag and Hoigne, 1983; von Gunten and Hoigne, 1994).

$O_3 + Br^- \rightarrow O_2 + OBr^-$	$k = 160 \pm 20\ M^{-1}\ s^{-1}$	(3.2.1)
$O_3 + OBr^- \rightarrow 2O_2 + Br^-$	$k = 330 \pm 60\ M^{-1}\ s^{-1}$	(3.2.2)
$OBr^- + H_3O^+ \rightarrow HOBr + H_2O$	$k = 2.06 \times 10^9\ M^{-1}\ s^{-1}$	(3.2.3)
$HOBr + H_2O \rightarrow OBr^- + H_3O^+$	$pK_a = 8.8 - 9.0$	(3.2.4)
$2O_3 + OBr^- \rightarrow 2O_2 + BrO_3^-$	$k = 100 \pm 20\ M^{-1}\ s^{-1}$	(3.2.5)
$HOBr + NH_3 \rightarrow NH_2Br + H_2O$	$k = 7.5 \times 10^7\ M^{-1}\ s^{-1}$	(3.2.6)

Haag and Hoigne (1983) and von Gunten and Pinkernell (2000) suggest that no reaction of HOBr with ozone occurs. The pK_a of 8.8 (the pH at which there exist equal amounts of HOBr and OBr^-) suggests that at normal seawater pH, a significant proportion of HOBr would be observed. Therefore, it is possible that "bromine" can accumulate in ozonated ballast water.

Crecelius (1979) studied the ozonation of seawater and measured the formation of bromate ion and total residual oxidant, TRO, (Figure 3.2.4). The concentration of both reaction products increased with the time of ozonation. The TRO showed an initial increase and subsequent decrease in concentration presumably due to back reactions as the reaction time increased. From the chemical cycle shown in Figure 3.2.3 it is likely that the measurement of TRO is entirely made up of HOBr/OBr^-. No oxidized forms of chlorine are possible under ozonation treatment conditions.

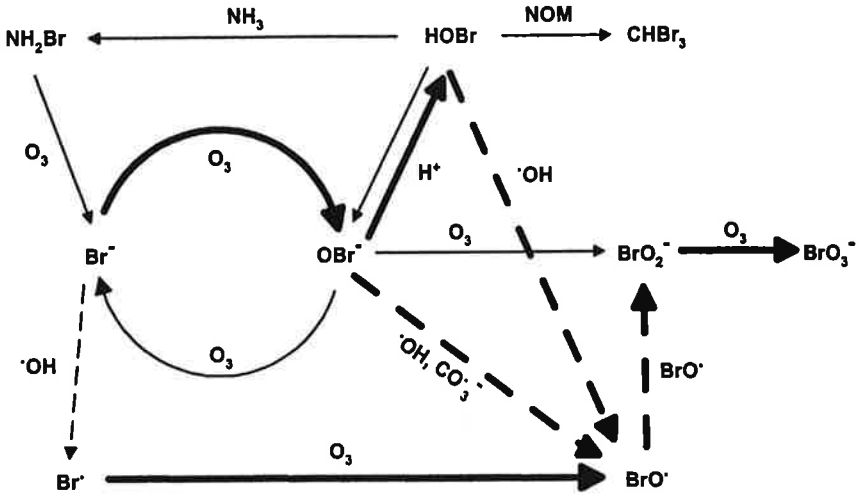

Figure 3.2.3. Reaction pathways for the decomposition of ozone in seawater with the formation of reaction by-products bromate ion and bromoform shown (Driedger et al., 2001 (Reprinted with permission from Elsevier Science); Haag and Hoigne, 1983; von Gunten and Hoigné, 1994; von Gunten and Oliveras, 1998).

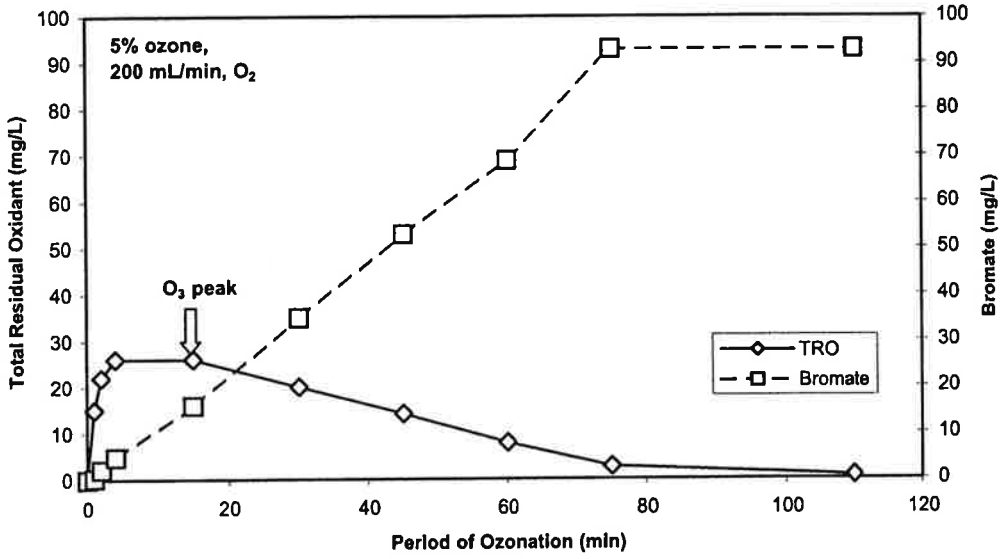

Figure 3.2.4. The ozonation of seawater with increasing time showing the formation of the reaction by-products, bromate ion and total residual oxidant (from Crecelius, 1979).

Therefore, any study designed to evaluate ozone as a possible treatment for ballast water using oceanic sources of water must include the analysis of the two oxidants (Table 3.2.1) and the two reaction by-products (Table 3.2.2).

Table 3.2.1. Oxidants measured when evaluating ozone for ballast water treatment.

Indicator	Definition	Potential significance for ballast water monitoring.
Ozone	O_3	Primary oxidant for ballast water treatment.
Bromine	$HOBr/OBr^-$	Results from the oxidation of bromide ion.

Table 3.2.2. Reaction by-products analyzed when evaluating ozone for ballast water treatment.

Indicator	Definition	Potential significance for ballast water monitoring.
Bromate ion	BrO_3^-	Results from the ozonation of bromide ion in salt water.
Bromoform	$CHBr_3$	Results from the reaction of bromine with naturally occurring organic mater in water used for ballast.

Oxidation-reduction reactions occur during the disinfection process. Thus, the ORP of ozonated water can provide an overall estimate of the oxidizing potential of the water. ORP has been used successfully in controlling ozone levels in aquaria (Aiken, 1995). This measurement may afford a control option for the ozone process. Aiken (1995) reports that for typical use of ozone in aquaria an ORP reading of 400 mV in seawater relates to an ozone dose of 0.02 mg/L, and a reading of 800 – 1000 mV inside the ozone contact chamber (of aquaria) would result in a water that was disinfected.

3.3 Toxicity of Ozone and Its By-products in Seawater

Ozone toxicity tests have been conducted for several marine taxa, including microalgae, invertebrates and vertebrates (Table 3.3.1). Unfortunately, the wide range of exposure conditions and test endpoints used among all of the marine toxicity tests makes it difficult to quantify a general effect concentration for ozone. Furthermore, analytical measurements taken in most tests were not specific to ozone, but rather are expressed as TRO, or "ozone-produced oxidants." "Ozone" toxicity is thus most correctly expressed as a function of TRO, rather than O_3 *per se.*

Many of the toxicity tests exposed organisms to ozone gas diffused in water for relatively short periods of time (e.g., 5-15 minutes), then measured acute toxicity over typical time periods (e.g., 24-96 hours). In these tests, substantial mortality (i.e., 50-100 % mortality) was observed for microalgae, crabs and lobster at concentrations ranging from 0.14 – 1.0 mg/L of TRO (Table 3.3.1). In most of these tests, TRO was measured using a standard amperometric titration reported as chlorine equivalents (Moffett and Shleser 1975, Toner and Brooks 1975). Crab zoea (free swimming planktonic crab larvae) and megalops (crab larval life stage after zoea stage) qualitatively were more sensitive to TRO than the microalgae or lobster, but no quantitative toxicity endpoints were derived in these tests.

Ozone toxicity tests with striped bass and white perch were conducted using flow-through test systems to deliver more reliable and consistent ozone exposures (Table 3.3.1; Block et al. 1978, Hall et al. 1981, Richardson et al. 1983). For striped bass, LC50s (i.e., concentration that kills 50 % of the organisms) ranged from 0.06 – 0.2 mg TRO/L depending on the life stage tested and length of exposure (Hall et al. 1981). Eggs were the most sensitive life stage when reared in freshwater (LC50 = 0.06 mg TRO/L), but fingerlings were most sensitive in seawater if the test was run for 96 hours (LC50 = 0.08 mg TRO/L). Slightly higher concentrations (0.15 – 0.4 mg TRO/L) induced 100% mortality (i.e., LC100) to striped bass fingerlings. In contrast to striped bass, TRO was slightly less toxic to white perch with LC50 values ranging from 0.2 – 0.38 mg TRO/L (Richardson et al. 1983), and an LC100 of 0.8 mg TRO/L after a 6-hour exposure (Block et al. 1978).

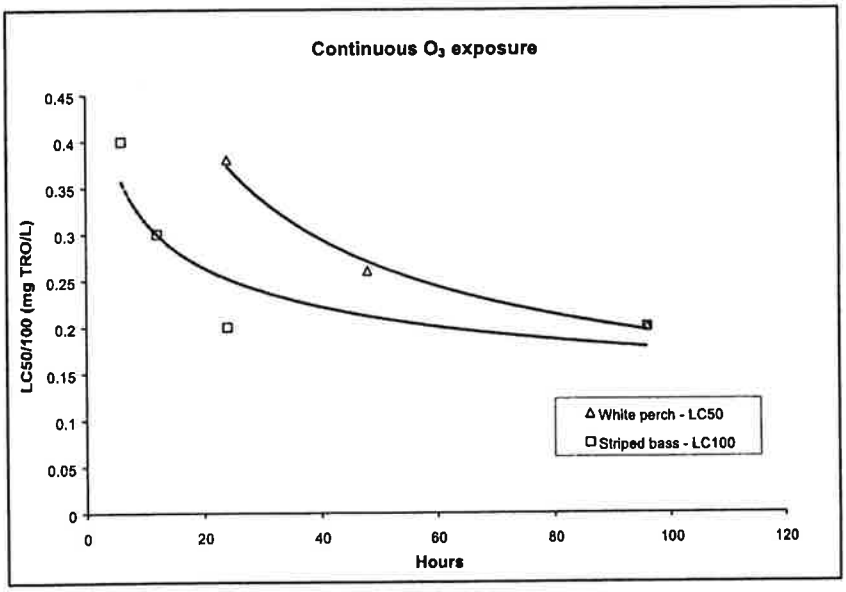

Figure 3.3.1: Toxicity of TRO to striped bass and white perch over time. Curves represent power functions fit to the data by species.

Like most contaminants, ozone toxicity is likely to increase as a function of increasing exposure time, although supporting data are scarce. While even short exposures (e.g., 5 minutes)

were sufficient to induce significant mortality in some organisms (Table 3.3.1), relatively high concentrations (e.g., 1 mg TRO/L) were sometimes required to induce this effect. The effect of time on ozone toxicity is perhaps best observed in 96 hours continuous exposures in which mortality was observed at various times throughout the tests. In experiments with striped bass fingerlings continuously exposed to ozone up to 96 hours, LC100 values decreased by 50% at 24-96 hours compared to that observed at 6 hours (Table 3.3.1; Figure 3.3.1). A similar relationship was observed for white perch adults, but using LC50 values rather than LC100 values with striped bass (Figure 3.3.1). Thus, ozone may be highly effective either via high concentration short-term exposures (e.g., 1 mg TRO/L for 5 minutes for microalgae), or via low concentration long-term exposures (e.g., 0.2 mg TRO/L for 96 hours for striped bass or white perch).

Given that TRO likely consists of bromine species in seawater (Section 7.3; Crecelius 1979), one would expect that bromine toxicity would be similar to ozone-generated TRO. Although the data are sparse, and most of that is for freshwater species, the literature confirms this expectation with LC50 values for fishes and invertebrates ranging from 0.015 – 1.5 mg bromine/L (Table 3.3.2). This further suggests that bromine may be the dominant ozone-produced residual oxidant of toxicological importance in seawater. Alternatively, bromine may be the only effective ozone-produced oxidant that persists long enough to have been measured in the toxicity tests conducted to date.

The most stable by-products of seawater ozonation typically are bromate ion and bromoform and both may persist long after ozone treatment is terminated (Section 3.2). However, the limited available toxicity data set suggests that these compounds are not acutely toxic with LC50 values 1 – 2 orders of magnitude higher than either TRO or bromine (Tables 3.3.3, 3.3.4). The most sensitive species to bromate ion is the mysid shrimp *Neomysis awatschensis* with an acute LC50 of 176 mg bromate ion/L, and the most sensitive species to bromoform is the sheepshead minnow with 96-hours LC50 values ranging from 7.1 – 18 mg bromoform/L. Therefore, even if bromate ion and/or bromoform are produced as by-products of seawater ozonation, they are not likely to be of toxicological concern (Section 7.3.4; see also Crecelius 1979).

Table 3.3.1. Toxicity of ozone to marine organisms (except as noted).

Species	Endpoint	Effect	Test type	Duration	TRO (mg/L)	Exposure Time	Notes	Reference
PHYTOPLANKTON								
Nannochloris sp.		growth / biomass	static	24 hr	0.45	10-15 min	severe biomass decrease in 24 hr after 10-15 min exposure	Toner and Brooks 1975
Nannochloris sp.		growth / biomass	static	72 hr	0.45	5 min	severe biomass decrease in ca. 72 hr after 5 min exposure, then recovers	Toner and Brooks 1975
Monochrysis lutheri		growth / biomass	static	48 hr	1.0	5 min	severe biomass decrease in ca. 48 hr after 5 min exposure	Toner and Brooks 1975
Skeletonema costatum		growth / biomass	static	24 hr	0.10	5 min	complete biomass depletion in 24 hr after 5 min exposure	Toner and Brooks 1975
INVERTEBRATES								
Homarus americanus (American lobster)		survival		6 d	0.40		no effect noted in larval survival or development to 2nd stage	Moffett and Shleser 1975
Crassostrea virginica (oyster)		"stress"	flow	1/2 d	1.0		no discernable adverse effect, in fact, it "improved handling characteristics"	Ciambrone 1975
Crab (species not identified)	0-20%	mortality		24 hr	0.080	1 min	zoea	Toner and Brooks 1975
Crab	0-20%	mortality		24 hr	0.080	1.5 min	zoea	Toner and Brooks 1975

Species	Endpoint	Effect	Test type	Duration	TRO (mg/L)	Exposure Time	Notes	Reference
Crab	40-70%	mortality		24 hr	0.14	5 min	zoea	Toner and Brooks 1975
Crab	100%	mortality		24 hr	0.329	10 min	zoea	Toner and Brooks 1975
Crab	30-40%	mortality		48 hr	0.080	1 min	zoea	Toner and Brooks 1975
Crab	50%	mortality		48 hr	0.080	1.5 min	zoea	Toner and Brooks 1975
Crab	80-90%	mortality		48 hr	0.140	5 min	zoea	Toner and Brooks 1975
Crab	100%	mortality		24 hr	0.20	1 min	megalops	Toner and Brooks 1975
Crab	100%	mortality		24 hr	0.20	2 min	megalops	Toner and Brooks 1975
FISHES								
Morone saxatilis (striped bass)	LC50[a]	mortality	flow	12 hr	0.21	continuous	eggs - estuarine water	Hall et al. 1981
Morone saxatilis	LC50	mortality	flow	30 hr	0.21	continuous	eggs - estuarine water	Hall et al. 1981
Morone saxatilis	LC50	mortality	flow	12 hr	0.060	continuous	eggs - freshwater	Hall et al. 1981
Morone saxatilis	LC50	mortality	flow	30 hr	0.060	continuous	eggs - freshwater	Hall et al. 1981
Morone saxatilis	LC50	mortality	flow	6 hr	0.15	continuous	larvae - estuarine water	Hall et al. 1981
Morone saxatilis	LC50	mortality	flow	96 hr	0.080	continuous	larvae - estuarine water	Hall et al. 1981
Morone saxatilis	LC50	mortality	flow	6 hr	0.20	continuous	fingerlings - estuarine water	Hall et al. 1981
Morone saxatilis	LC50	mortality	flow	96 hr	0.080	continuous	fingerlings - estuarine water	Hall et al. 1981
Morone saxatilis (larvae)	LC100[b]	mortality	flow	6 - 96 hr	0.15	continuous	larvae - estuarine water. LC100 = lowest concentration where complete mortality observed (no stats)	Hall et al. 1981

Morone saxatilis (fingerling)	LC100	mortality	flow	6hr	0.40	continuous	fingerlings - estuarine water	Hall et al. 1981
Morone saxatilis (fingerling)	LC100	mortality	flow	12 hr	0.30	continuous	fingerlings - estuarine water	Hall et al. 1981
Morone saxatilis (fingerling)	LC100	mortality	flow	24-96 hr	0.20	continuous	fingerlings - estuarine water	Hall et al. 1981
Morone americana (white perch)	LC100	mortality	flow	6 hr	0.80	continuous		Block et al. 1978
Morone americana	LOEC[c]	blood pH	flow	1 hr	0.80	continuous		Block et al. 1978
Morone americana	LOEC	blood pOsm	flow	3 hr	0.80	continuous		Block et al. 1978
Morone americana	LOEC	blood pCl	flow	3 hr	0.80	continuous		Block et al. 1978
Morone americana	LOEC	blood pK	flow	2 hr	0.80	continuous		Block et al. 1978
Morone americana	LOEC	blood pMg	flow	1 hr	0.80	continuous		Block et al. 1978
Morone americana	LOEC	blood pCa	flow	1 hr	0.80	continuous		Block et al. 1978
Morone americana	LOEC	gill protein	flow	5 hr	0.80	continuous		Block et al. 1978
Morone americana	LC50	mortality	flow	24 hr	0.38	continuous		Richardson et al. 1983
Morone americana	LC50	mortality	flow	48 hr	0.26	continuous		Richardson et al. 1983
Morone americana	LC50	mortality	flow	96 hr	0.20	continuous		Richardson et al. 1983
Menidia menidia (silverside minnow)		100% mortality		30 min	0.14	5 min	25% control mortality after 24 hr	Toner and Brooks 1975

[a]LC50 = 50% lethal concentration. Concentration of chemical that causes 50% mortality in an acute toxicity test.
[b]LC100 = 100% lethal concentration. Concentration of chemical that causes 100% mortality in an acute toxicity test.
[c]LOEC = Lowest observed effect concentration. Lowest exposure concentration which a statistically significant adverse effect was observed.

Table 3.3.2. Toxicity of bromine to aquatic organisms.

Species	Endpoint	Effect	Test type	Duration	Conc. (mg/L)	Reference
INVERTEBRATES						
Daphnia magna (water flea)	LC50[a]	mortality	static	24 hr	1.5	LeBlanc 1980
Daphnia magna	LC50	mortality	static	48 hr	1	LeBlanc 1980
FISHES						
Lepomis macrochirus (bluegill sunfish)	LC50	mortality	static	24 hr	0.52	USEPA 1995a
Oncorhynchus mykiss (rainbow trout)	LC50	mortality	static	24 hr	0.31	USEPA 1995a
[1] *Strongylocentrotus droebachiensis* (green sea urchin)	EC50[b]	reproduction	static	5 hr	0.015	Dinnel et al. 1981

[a]LC50 = 50% lethal concentration.
[b]EC50 = 50% effect concentration. Concentration which an absolute test endpoint value is 50% of the absolute value in the controls.
[1] Marine species

Table 3.3.3. Toxicity of bromate ion to marine organisms.

Species	Endpoint	Effect	Test type	Duration	Bromate (mg/L)	Reference
INVERTEBRATES						
Crassostrea gigas (oyster)	EC50[a]	develop-ment	static	2 d	30	Crecelius 1979
Neomysis awatschensis (mysid shrimp)	LC50[b]	mortality	static	1 d	176	Crecelius 1979
Macoma inquinata (bentnosed clam)	LC100[c]	mortality	static	3 d	880	Crecelius 1979
Pandalus danae (connstripe shrimp)	LC100	mortality	static	3 d	880	Crecelius 1979
Protothaca staminea, (littleneck clam)	LC100	mortality	static	3 d	880	Crecelius 1979
FISHES						
Oncorhynchus keta (chum salmon)	LC50	mortality	static	4d	512	Crecelius 1979
Cymatogaster aggregata, (shiner perch)	LC100	mortality	static	3d	880	Crecelius 1979

[a]EC50 = 50% effect concentration. Concentration which an absolute test endpoint value is 50% of the absolute value in the controls.
[b]LC50 = 50% lethal concentration.
[c]LC100 = 100% lethal concentration.

Table 3.3.4. Toxicity of bromoform to marine organisms.

Species	Endpoint	Effect	Test type	Duration	Bromoform (mg/L)	Reference
Phytoplankton						
Skeletonema costatum	IC50[a]	growth	static?	7 d	32	Erickson and Freeman 1978
Thalassiosira pseudonana	IC50	growth	static?	7 d	32	Erickson and Freeman 1978
Glenodinium halli	IC50	growth	static?	7 d	32	Erickson and Freeman 1978
Isochrysis galbana	IC50	growth	static?	7 d	32	Erickson and Freeman 1978
INVERTEBRATES						
Americamysis bahia (mysid shrimp)	LC50[b]	mortality	flow	4 d	24.4	USEPA 1978
Penaeus aztecus (brown shrimp)	LC50	mortality	flow	4 d	26	Anderson et al. 1979
FISHES						
Brevoortia tyrannus (Atlantic menhaden)	LC50	mortality	flow	4 d	12	Anderson et al. 1979
Cyprinodon variegatus (sheepshead minnow)	LC50	mortality	static	1 d	19	Heitmuller et al. 1981
Cyprinodon variegatus	LC50	mortality	static	2 d	19	Heitmuller et al. 1981
Cyprinodon variegatus	LC50	mortality	static	3 d	18	Heitmuller et al. 1981
Cyprinodon variegatus	LC50	mortality	static	4d	18	Heitmuller et al. 1981
Cyprinodon variegatus	LC50	mortality	flow	4d	7.1	Ward et al. 1981
Cyprinodon variegatus	NOEC[c]	juv.mort.	flow	28 d	4.8	Ward et al. 1981
Cyprinodon variegatus	LOEC[d]	juv.mort.	flow	28 d	8.5	Ward et al. 1981

[a]IC50 = 50% inhibition concentration. Concentration which a test endpoint is inhibited by 50% compared to controls.
[b]LC50 = 50% lethal concentration.
[c]NOEC = No observed effect concentration.
[d]LOEC = Lowest observed effect concentration.

4 EXPERIMENTAL SYSTEM

4.1 The *S/T Tonsina*

The *S/T Tonsina* is an 869-foot American-flagged oil tanker operated by Oregon-based Alaska Tanker Company in what is commonly known as the TAPS (Trans Alaskan Pipeline Service) trade of Alaska North Slope crude oil. This oil is transported mainly between Valdez, Alaska and refineries on the west coast of the United States. The *S/T Tonsina* can carry 270,000 barrels of ballast water, or more than 11 million gallons (41,600,000 L) in its 12 ballast water tanks, and 807,000 barrels (nearly 34 million gallons) of crude oil in its 12 cargo tanks.

The *S/T Tonsina* has a double hull, which means that the cargo tanks are protected by an outer hull, and the space between the hulls is divided transversely into segregated sections for carrying ballast water when the ship is empty or only partially loaded. These ballast tanks are arranged along the vessels' outer hull and double bottom area (see Figure 4.1.1). Although each wing tank area is connected to the double bottom tank area, water circulation between these two areas is believed to be poor.

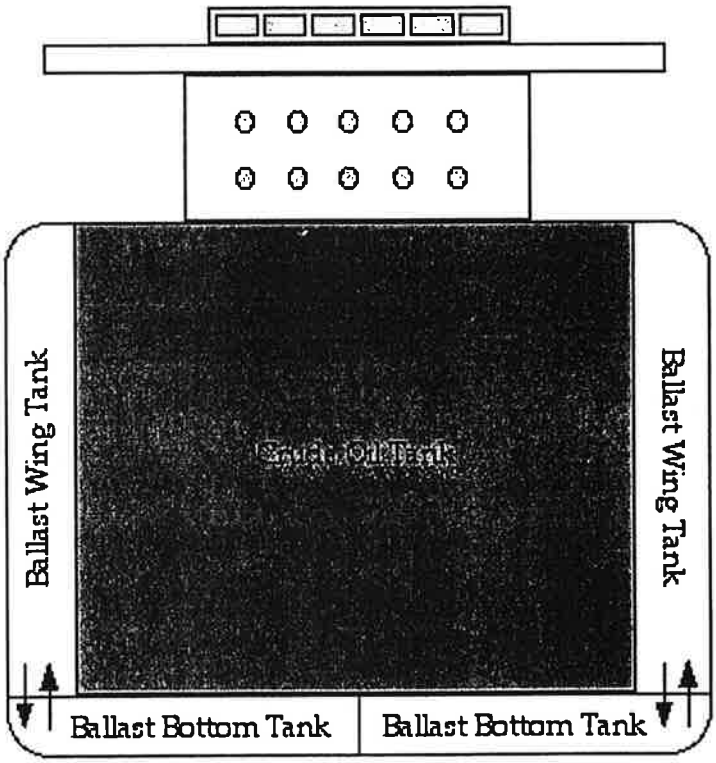

Figure 4.1.1. Cross section (not to scale) of the *S/T Tonsina's* ballast tank arrangement. The arrows signify the presence of some circulation between the wing and bottom tanks, but the extent of this circulation is believed to be limited.

4.2 The Ozone System

In the fall of 2000, a prototype Nutech ozonation system was installed on the *S/T Tonsina* during a planned out-of-service period, while afloat at the Hyundai Mipo Drydocks in Ulsan, South Korea. The customized prototype, known as the SCX 2000, was built to fit inside a standard ISO 20 foot container in order to facilitate the installation. The container was installed on the *S/T Tonsina's* stack deck, in an exterior location.

Ozone is produced by sending a stream of oxygen-enriched compressed air through a series of water-cooled electrodes. Within each electrode, a high voltage corona discharge is created (an electric arc), using a standard ship's 480-volt power transformed to more than 10,000 volts. As the oxygen enriched air stream passes through each corona gap, a percentage of the air stream is converted into ozone, which is then collected and piped into each of the 12 ballast tanks, through a system of flow meters and stainless steel pipe. The ozone is distributed throughout each ballast tank by a system of 1,200 custom designed ceramic coated stone diffusers, arranged to maximize the distribution and contact time of the ozone. Ballast tanks can be ozonated individually or in groups, with the prototype's maximum system capacity of 1800 gm O_3/hour, leading to an O_3 loading rate of ca. 0.6 mg/L/hour in each tank when treated individually.

Because the *S/T Tonsina* is double-hulled, its ballast tanks are between the hulls that surround the ship's central oil cargo tanks and are separated into a series of baffled chambers. The chambers are interconnected vertically and horizontally by openings large enough for maintenance personnel to pass through. At the top of each series of chambers is either a manhole for personnel access or an approximately 12-inch Butterworth® hatch used for the deployment of cleaning equipment (Figure 4.2.1).

The ozone gas diffusers were arranged in 8 rows running horizontally with the beam of the vessel, with 7 rows placed in the double bottom section of the ballast tank (underneath the oil cargo tank), and 1 row placed at the bottom of the vertical side tank (Figure 4.2.2), in the curve of the bilge area.

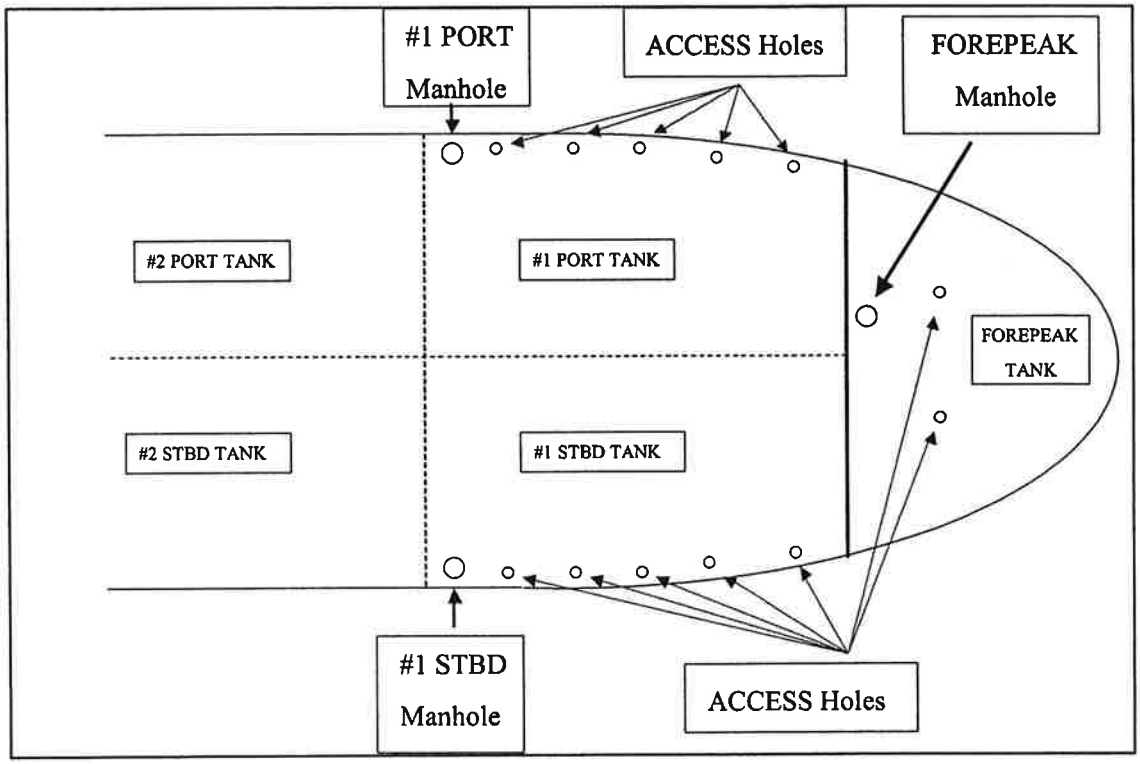

Figure 4.2.1. Top view of access hatches on deck of S/T *Tonsina*

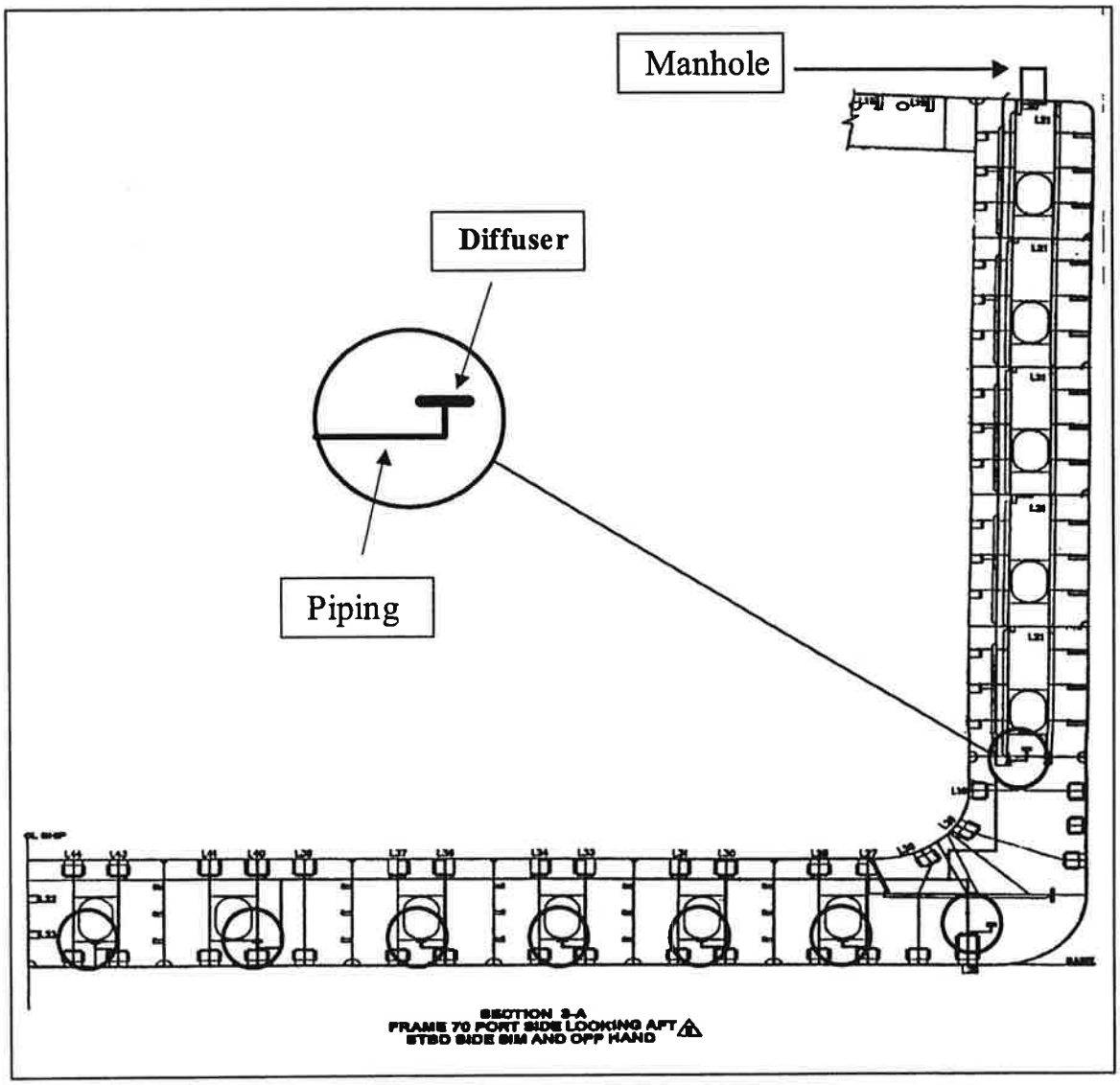

Figure 4.2.2. Layout of ozone diffusers shown in cross section of the ballast tanks on the *S/T Tonsina.*

5 PRELIMINARY STUDIES

Preliminary studies of this system were undertaken in November 2000 on board the *S/T Tonsina*. The experimental design was relatively simple. The two No. 3 ballast wing tanks (port and starboard) were studied. The port was the control to which no ozone was added and the starboard was ozonated. Samples were withdrawn immediately prior to the initiation of the test (at 0 hours) and then at 2, 4, 6, 8 and 12 hours after ozonation or oxygen bubbling (for the control tank). There were 10 sample lines in each tank. The samples were drawn from several depths in each tank (Figure 5.1.1). In addition to bacterial counts, bromate and bromoform concentrations were measured.

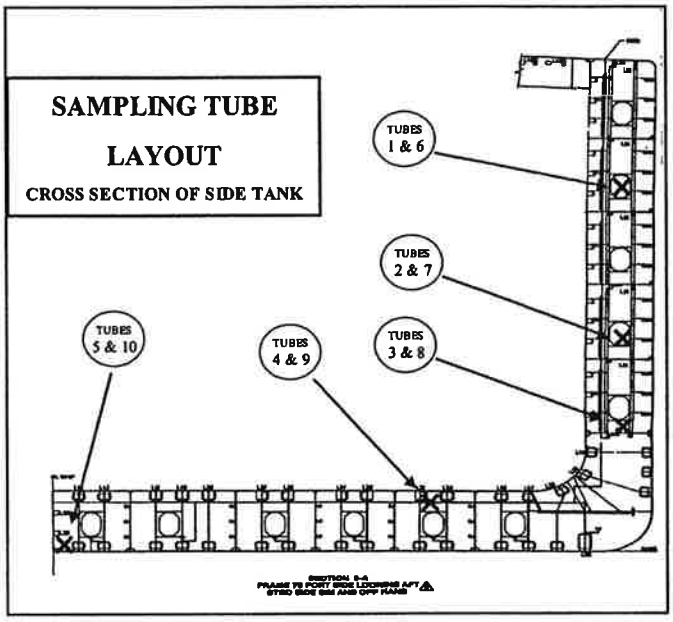

Figure 5.1.1. Sample Tube Locations

The results are summarized below for the bacterial kill (Table 5.1.1) and bromoform formation (Table 5.1.2). There was no bromate ion found (detection limit of 1 μg/L) in any of the samples.

Table 5.1.1. Summary of the bacterial numbers (direct count) in the ozone treated ballast tank, 3S.

Sample Line	Time (hours)					
	0	2	4	6	8	12
1	6.9×10^4	1.9×10^4	2.3×10^3	3.6×10^2	4.4×10^2	5.6×10^2
2	1.1×10^5	3.6×10^3	7.8×10^2	5.1×10^2	8.5×10^2	3.3×10^2
3	3.1×10^3	5.3×10^3	1.5×10^3	3.5×10^2	2.0×10^2	1.5×10^2
4	4.3×10^3	1.2×10^3	1.5×10^2	1.5×10^2	3.6×10^1	3.6×10^1
5	3.7×10^3	5.6×10^2	1.3×10^2	9.1×10^1	9.1×10^1	9.1×10^1
6	6.4×10^3	2.6×10^3	7.7×10^2	5.4×10^2	4.1×10^2	3.2×10^2
7	5.2×10^4	2.1×10^3	5.0×10^2	1.8×10^2	4.4×10^2	3.6×10^2
8	9.3×10^3	4.7×10^2	2.4×10^2	9.3×10^2	6.4×10^2	1.8×10^2
9	1.4×10^4	2.3×10^4	1.5×10^3	3.6×10^2	5.0×10^2	3.6×10^2
10	6.9×10^4	3.8×10^4	1.5×10^3	3.3×10^2	1.1×10^2	1.6×10^2

Table 5.1.2. Summary of the bromoform concentration (µg/L) in the ozone treated ballast tank, 3S.						
Sample Line	Time (hours)					
	0	2	4	6	8	12
1	BMDL[1]	11.2	NA[2]	117.9	174.9	171.6
2	BMDL	112.8	NA	148.8	89.8	NA
3	BMDL	53.3	111.1	89.6	172	158.5
4	BMDL	47	112.2	121.3	147.4	164.2
5	BMDL	83.9	133.7	161.3	321.8	255.5
6	BMDL	61.1	138.9	137.4	154.4	207.8
7	BMDL	130.5	153.4	145.4	179.1	215.1
8	BMDL	112	142.3	178.7	177.9	294.2
9	BMDL	42.3	98.6	115.4	161.6	168.4
10	BMDL	9.6	NA	NA	NA	177.2
1 BMDL = below method detection limit 2 NA = not analyzed						

A summary of the relationship of bromoform formation and bacterial kill is shown (plotted as arithmetic means for all 10 sample lines) in Figure 5.1.2.

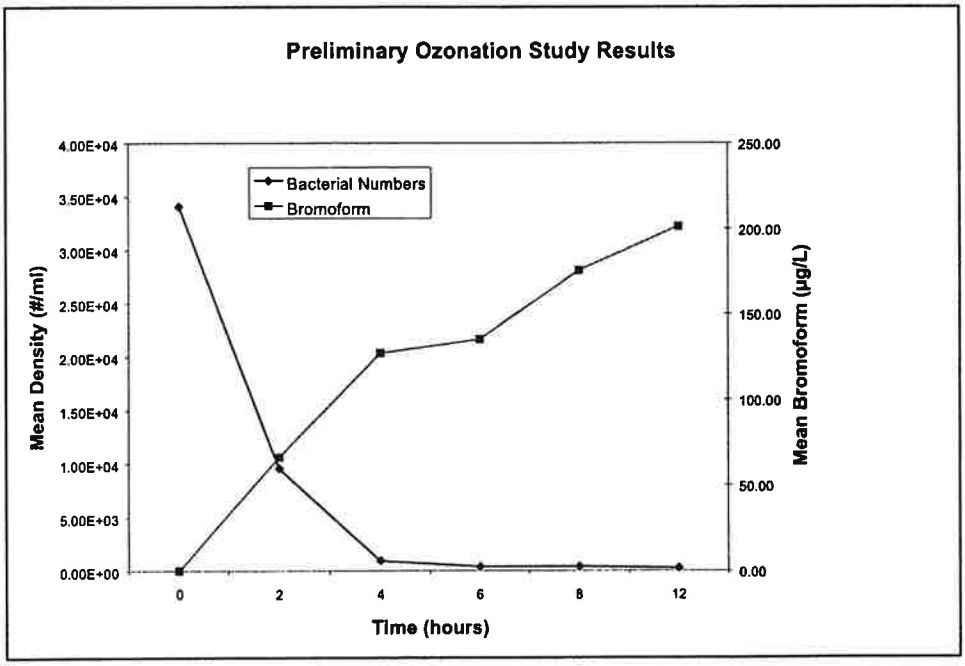

Figure 5.1.2. Plot of bacterial numbers and bromoform concentrations over time

The results showed that the *S/T Tonsina's* ozone treatment system achieved, on average, 99% removal of the bacteria after four to six hours. The bromoform concentration varied somewhat but was in general slightly higher than 100 µg/L at the time 99 % bacterial removal

was observed. These data suggest that ozonation was highly effective in removal of free-living bacterial concentrations. While this occurred simultaneously with the generation of bromoform as a reaction by-product, it is doubtful that these bromoform concentrations would be high enough to cause a direct toxic impact. Rather, it is likely that ozone and its reactants (Table 3.2.1 and 3.2.2) may have been most responsible for reductions in bacterial numbers.

6 MATERIALS AND METHODS

6.1 Overview

On four occasions, the 13-member experimental team assembled to conduct ozone experiments aboard the *S/T Tonsina* while she was anchored at Port Angeles in Washington State. This occurred once in May, once in September and twice in November of 2001. In May, the ozone experiment was terminated after less than two hours of ozonation because the electrical transformers that provide power to the ozone generator began to overheat and had to be shut down to prevent further damage to the system. Although this prevented the collection of any ozone-related data, the experimental team elected to follow through as scheduled with the BWE portion of the experiment during the return voyage to Valdez, Alaska. It took several months before the ozone generator was repaired and the system was again available for testing.

On September 24, a five-hour ozonation experiment was successfully conducted (Experiment 1). Following review of the data from that experiment, the team decided to change the exposure period to 10 hours for the final two experiments, which were held on November 2 (Experiment 2) and November 4 (Experiment 3). The second and final BWE experiment was conducted in September following the first successful ozone experiment. BWE experiments were not conducted following the second and third ozone experiments in November because the *S/T Tonsina* did not sail into open ocean following these experiments but rather sailed to Portland, Oregon, for repairs and temporary lay-up.

In all three-ozone experiments, the No. 3 port wing ballast tank was used for the ozone treatment tank, and the No. 3 starboard wing ballast tank was used for the control tank. These are both tall, vertically oriented tanks that were sampled from several access points on the ship's deck. Five-liter Niskin bottles were used to collect water samples at three depths from these tanks, and sub samples for chemistry, bacteria and phytoplankton were collected from these bottles and processed into the appropriate containers and were analyzed using methods described below. A zooplankton net was used to collect zooplankton samples from the tank, and these samples were immediately examined under a microscope on board the ship. Caged organisms were also deployed at four depths in both tanks and the kill ratio was established by determining the live/dead/moribund status following the ozonation exposure period as described below.

Each sample type was collected from two access points above each tank to help assess some amount of spatial variability in ozone system efficacy. Each tank was sampled in two sections (i.e.,columns), with the forward and rear portion of the treatment tank referred to as Column A and Column B, respectively, and the forward and rear portion of the control tank referred to as Column C and Column D (See Figure 6.1.1). Since Niskin bottle samples were collected from three depths at each column at each time point, this gave rise to a letter/number sample labeling where the letter signified a time point (T) or column location (A, B, C, D) and the number signifies either the time or depth (in feet) from the surface at which it was taken. For example, sample T-0.0B50 represents the sample at the initial time (T) of the experiment (0.0), before the ozone generator was engaged, and the column (B) and depth (50 feet) at which it was taken. Another example would be T-7.5A10, which would represent the sample time of 7.5 hours from the beginning of ozone inundation at the depth 10 feet from the surface in Column A.

In Experiment 2 and Experiment 3, Column D was dropped due to the increased samples collected with additional time points. Column C then became the only control tank column and was moved from the front to the middle of the tank.

As each filled Niskin bottle was brought to the surface, a single water quality sample was collected to measure pH, salinity, dissolved oxygen, and oxidation-reduction potential. Duplicate samples were then collected into specimen cups for immediate analysis of both ozone and TRO (Section 6.3.2). Ozone Accu-vacs were used for the ozone spectrometric analysis and total chlorine Accu-vacs were used for TRO analysis. These analyses were made using a portable analysis kit (DREL with a DR/2010 spectrometer manufactured by the Hach Company, Loveland, CO), which was set up on board the tanker.

From these same Niskin casts, samples were collected and immediately placed on ice for later transport to the respective analytical laboratories: samples for bacteria analysis were collected into one-liter polypropylene bottles, samples for phytoplankton analysis were collected into one-liter HDPE bottles, samples for bromate ion analysis were collected in 50 ml HDPE bottles, and duplicate samples for bromoform were collected in 40 mL glass amber VOA vials (Section 6.3).

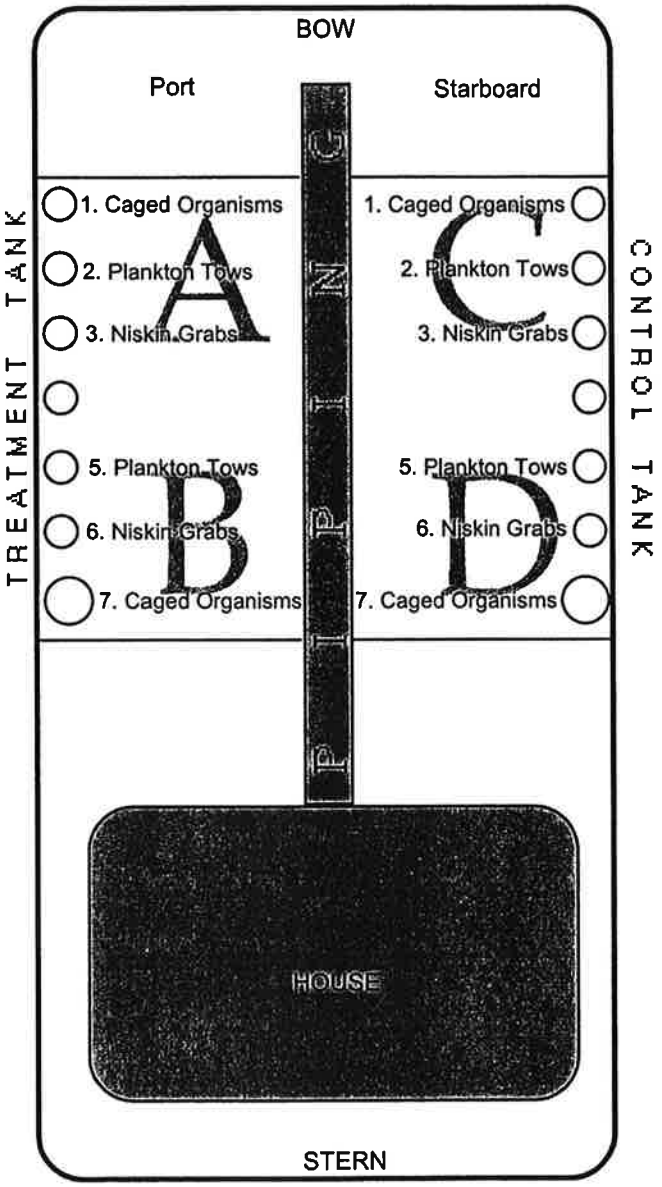

Figure 6.1.1. Schematic of the layout of No. 3 port and starboard ballast tanks on deck. The seven access points to each wing tank are shown, along with corresponding samples types. Drawing is not to scale, as there are also several other such ballast wing tanks on each side of the ship. Note that Column D was not sampled in Experiments 2 and 3.

6.2 Ozone Delivery

The ozone system installed on the *S/T Tonsina* is capable of delivering ozone to each vertical wing tank and horizontal bottom tank, even though these are interconnected, making the two tanks essentially two sections of one L-shaped tank. However, as mentioned earlier, circulation between these two sections is believed to be poor, thus, results are likely to occur as if they were completely separate tanks with respect to the movement of water or organisms.

The ability to distribute ozone between the two sections was examined in the three experiments. Although this diminished replication between experiments, varying the ozone delivery revealed how the chemistry and biology in the tank responded to different ozone-loading rates.

In Experiment 1, ozone delivery between the vertical and horizontal section was evenly divided, with 50% going to each section. In Experiment 2, 60 percent of the ozone was delivered to the vertical section (which was the section sampled during the experiments) with the remaining 40% delivered to the bottom horizontal section. In Experiment 3, 100 percent of the ozone was delivered to the vertical section and none to the horizontal section. The most effective biological kill ratios were seen in this last experiment.

6.3 Water Chemistry

6.3.1 Water Quality

Water quality analyses were conducted on board ship using a Hach DREL/2010 Water Quality Laboratory and nutrients were later analyzed in a laboratory. All water quality samples were obtained from Niskin bottle grabs, and analyzed according to the instructions provided with the Hach water quality test kit. The determination of pH was conducted using a Hach Portable pH Meter (Hach Company, Loveland, CO). Dissolved oxygen was measured with a model 21800-022 Traceable® digital dissolved oxygen meter. This meter was air calibrated and adjusted to compensate for salinity. Salinity was measured using a conductivity meter with a range of 0-80 ‰ (Hach Company, Loveland, CO). Temperature was determined using a standard field thermometer. Inorganic nutrients (ortho-phosphate, nitrite, nitrate, ammonia, silicic acid) were analyzed from stored, refrigerated samples at the University of Washington using standard colorimetric techniques.

6.3.2 Ozone Chemistry

Total Residual Oxidant (TRO): TRO was determined using a standard DPD colorimetric analysis for total chlorine (APHA 1998). Samples were collected and KI was added and the TRO determined spectrophotometrically in the range 0-4.5 mg/L as Cl_2. This was achieved by using Hach® brand Accu-vac vacuum reaction containers, which were submerged and filled with ballast water samples immediately after the samples were collected from the tank. The filled Accu-vac containers were analyzed on a Hach® DREL 2010 spectrometer water quality lab kit.

Ozone: The presence of ozone was measured using an Indigo colorimetric technique (APHA 1998). Similar to TRO, Accu-vacs reaction containers were used with fresh Niskin grab samples and analyzed by using a DREL 2010 kit.

Oxidation Reduction Potential (ORP): Oxidation-reduction potential was measured by using an Orion 290A pH meter with a Cole-Palmer Combination ORP probe (Pt electrode, Ag/AgCl reference cell). In Experiments 2 and 3, additional ORP measurements were read directly inside ballast water tanks using a Hydrolab (Austin, TX) Quanta monitoring system that included an Ag/AgCl ORP sensor (see Section 7.7.4).

Bromate ion: Samples for bromate ion analysis were collected in 150 mL wide-mouth HDPE bottles. These were stored on ice and shipped to analytical laboratories as soon as possible after completion of the study. Two ion chromatography methods are available for measuring bromate ion: USEPA Method 300.1 and USEPA Method 317. Method 300.1 employs conductivity detection while Method 317 uses a post-column detection procedure to overcome interferences that may occur using Method 300.1. For seawater with higher ionic strength, when the chloride ion can potentially interfere in Method 300.1, Method 317 (EPA Document # EPA 815-B-01-001; http://www.epa.gov/safewater/methods/sourcalt.html) was used.

These methods incorporate chlorpromazine reaction chemistry to measure low-level bromate ions (BrO_3^-) following the separation of anions. The post-column procedure uses photometric detection at 530 nm (nanometers). Following separation, BrO_3^- reacts with chlorpromazine in acidic media by a charge transfer mechanism. This method overcomes well-known problems encountered in the ion chromatographic measurement of BrO_3^- from Cl^- and CO_3^{2-}. This procedure shows no interference from all common anions with the exception of chlorite ion and nitrite ion. This method can be used to measure bromate ion from 3 to 40 µg/L. The method detection limit (MDL) was determined to be 2-3 µg/L bromate ion.

For Experiment 1, the samples were shown to have a bromate ion concentration less than 2 µg/L. During the first set of analyses, it was observed that QA samples spiked with bromate ion were "unrecoverable." The subsequent evaluation of bromate ion standards prepared in distilled water (i.e., standards) showed good recovery.

Thus, studies were conducted to ascertain why spiked bromate ion in the ballast water samples could not be recovered. These studies showed that at higher concentrations (i.e., at the mg/L level), spiked bromate ion could be recovered. Subsequently, all of the ballast water samples were diluted to 20 % of their original concentration (1:5 dilution). With this dilution, it was determined that adequate bromate ion recovery could be achieved at the 50 ppb level.

Based on the bromate ion recovery following a 1:5 dilution, all of the ballast water samples for experiments 2 and 3 were diluted. Because the detection limit of the method is approximately 2 µg/L bromate ion, this dilution would still enable the detection of 10 µg/L bromate ion, which is the MCL established for bromate ion in drinking water.

Bromoform: Samples for bromoform analysis were collected in 40-mL VOA vials and were stored on ice and shipped to the analytical laboratory as soon as possible after completion of the study. The maximum acceptable sample analysis holding time is 14 days after sample collection. Bromoform was analyzed using a purge and trap (dynamic stripping) system coupled to a Hewlett Packard Model 5890 Series II gas chromatograph. The chromatograph was equipped with a 30 meter VOCOL capillary column, HP 3396A integrator/printer, and flame ionization detector. Bromoform was obtained from Ultra Scientific (product #HC-020, 100 ng/μL $CHBr_3$, Lot # R-1194 Standard Reference Material (SRM) traceable to the National Institute for Standards and Testing). Tekmar Model LSC-2000 Liquid Sample Concentrator interfaced with a Tekmar Model 2016 Autosampler system. Ultra pure Carrier-grade helium gas was used for sparging samples.

Initial calibration and calibration verification checks were preformed using known amounts of SRM prepared within laboratory-purified water. The standards used were with concentrations of 5, 10, 20, 50, 100 and 200 μg/L. Each solution was analyzed and the average instrument response factor was calculated by dividing the area counts observed for each standard solution.

Quantities of 5.0 ml were sub-sampled from the field sample bottle by using a gas tight syringe, after 200 ng of surrogate standard, a,a,a-trifluorotoluene was added to the sub-samples. Samples were sparged with helium gas for 12 minutes at a rate of 30 mL/minutes onto a Tenax trap at ambient temperature (less than 25 °C). After completion of the sparge cycle, the sample was desorbed from the Tenax trap at 250 °C for two minutes. The sample was transferred to the gas chromatograph splitless inlet using a heated nickel transfer line. After the transfer was completed, the Tenax trap was baked at greater than 250 °C for eight minutes between samples. The chromatogram was recorded on the HP 3396A integrator by setting it at the proper sensitivity to produce peak height of the surrogate compound to greater than 50% full scale. The sample location, date, time and sample volume were recorded for each analysis on the integrator printout and within the bound laboratory GC Logbook.

Initial GC external standard calibrations were conducted by preparing a multipoint instrument calibration by injecting a range of volumes of the $CHBr_3$ SRM into 5 mL of laboratory purified water. Each solution was analyzed and a calibration curve plotted. A linear regression coefficient for the SRM concentrations was determined. If the regression coefficient was greater than 0.997, the calibration was acceptable for the range of concentrations analyzed.

A calibration verification check sample (CVCS) with an SRM concentration equivalent to approximately 50% of the highest standard solution was analyzed twice each day whenever process samples were analyzed: once prior to the first process sample analysis, and once at the end of the day's analytical batch. The percent recovery was calculated by dividing the actual concentrations of bromoform detected by the theoretical concentration of the CVCS standard analyzed and multiplying by 100 %. If the calculated recovery was below 50% or greater than 150%, the CVCS standard for that analytical batch was unacceptable and the CVCS was reanalyzed and a new calibration curve was determined.

Two calibration verification standards were analyzed per day. The percent recovery of each compound was calculated and recorded on the quality control chart. A sample was analyzed in duplicate once per week and the relative percent difference (RPD) for the detected concentration in the process sample was determined. This was achieved by dividing the range of the detected concentrations by the mean of concentrations and multiplying by 100 %. A RPD of less than 30 % was considered to be acceptable. In addition, all method blank analyses with each batch of process samples were preformed. Target bromoform detections equal to or greater than two times the method detection limit were considered non-compliant. For non-compliant tests, appropriate corrective action was performed and each affected analysis was repeated.

6.4 BWE Experiments

The exchange experiments were conducted using *S/T Tonsina* protocols for open ocean exchange. A simpler version of the ozonation experimental design was used in this experiment. Only a limited crew (two or three) rode the *S/T Tonsina* on her return to Valdez carrying the same ballast water as was used in the ozonation experiment. This was done once in May following an aborted ozonation experiment (due to electrical problems with the ozone generator), and once in September following the five-hour ozonation experiment. Niskin grab samples were collected for simple water chemistry (e.g., pH, DO, nutrients) and microbial and plankton community composition were determined. Net tow samples provided organisms for the zooplantkton analysis.

The sampling design is summarized in Table 6.4.1. Both of these experiments used the same control tank as the ozone experiments, the No. 3 starboard wing ballast tank. The treatment tank that underwent BWE was the No. 4 port wing ballast tank, a tank adjacent and nearly identical to the ozone treatment tank (No. 3 port). For each of the two ballast exchange experiments, the BWE tank (No. 4 port wing) was sampled prior to the ozone experiment, prior to the exchange experiment, and after the exchange experiment. The type of exchange for the May experiment was a 100 % empty/refill, while the September experiment was 200 % empty/refill.

Both the control and exchange tanks were sampled with a zooplankton net identical to that used in the ozone experiments, and these samples were fixed for later microscopic analysis. Both tanks were also sampled using a five-liter Niskin bottle that provided sample for phytoplankton and chemistry analysis.

Table 6.4.1. Summary of the sampling schedule for the BWE experiment, with the number of samples *per ballast tank* indicated.

Time	Niskin grabs	Zooplankton Tows
	Phytoplankton	
Pre-ozonation	2 columns x 2 depths x 2 reps. (8)	2 columns x 2 depths (4)
Pre-exchange	8	4
Post-exchange	8	4
Totals	**24**	**12 tows**

6.5 Whole Effluent Toxicity (WET) Testing

Ballast water handling procedures ultimately require the discharge of ballast water into the open-ocean or estuarine waters. "Active" treatment technologies (e.g., addition of chemical sterilants or ozonation) that could result in the formation or introduction of toxic materials in ballast water are likely to come under some degree of regulatory scrutiny to ensure that the discharge of "toxic waters in toxic amounts" (Clean Water Act) does not occur. Vessel operators will likely be required to provide evidence that no adverse effects to organisms in the receiving water will result from the discharge of the treated ballast water. Confirmation of this is likely to be similar to the requirements currently used for monitoring the discharge of permitted point-source effluents via the National Pollutant Discharge Elimination System (NPDES), i.e., a combination of chemical specific measurements and whole effluent toxicity (WET) tests.

In order to see how ozone-treated waters would respond to these tests, samples of ozone-treated ballast waters were submitted for laboratory toxicity testing, using the same methods employed in conducting WET tests. Two standard marine toxicity tests were performed with water samples from post-ozonation ballast-water tanks: 1) the mysid shrimp (*Americamysis bahia*) static acute toxicity test, and 2) the topsmelt (*Atherinops affinis*) static acute toxicity test. These species have been shown to be among the most sensitive organisms when exposed to toxic chemicals in seawater (Suter and Rosen, 1988), and are considered to be suitable surrogates for indigenous species. Both tests are commonly used to evaluate the toxicity of effluents discharged into marine waters.

All toxicity tests were performed in accordance with standard procedures developed by the U.S. Environmental Protection Agency (U.S. EPA 1993, 1999). The seawater used as experimental controls and for dilution of ballast water samples was prepared using laboratory freshwater (1 μm filtered) and commercially available seawater salts (Hawaiian Marine Mix). The seawater strength was 30 ± 2 ‰ salinity. Ballast water samples (from both the ozone-treated and the un-treated control ballast tanks) were collected during each of the three field trials on 24 September 2001 (Experiment 1), 2 November 2001 (Experiment 2), and 4 November 2001 (Experiment 3). Samples were transported as soon as possible (within 24-48 hrs while stored at <4 °C) to the Parametrix, Inc. toxicology laboratory (Kirkland, WA) for testing; all laboratory tests were initiated within 24 hours of sample receipt.

Mysid shrimp were obtained from a commercial supplier (Aquatic Biosystems, Inc., Fort Collins, CO). Mysids (5 days old at the time of test initiation) were exposed for 48 hours in a static test to five dilutions of ballast water: 6.25, 12.5, 25, 50, and 100% ballast water and to a dilution water control. Organisms were maintained at a water temperature of 25 ± 1°C under a 16:8 hour light:dark cycle. Test solutions were not aerated and mysids were not fed during the tests. Four replicate test solutions containing five to ten animals per chamber were used at each treatment level in all tests. Organisms were monitored for survival/mortality daily over the

course of the test and at the end of the test the results were used to determine median lethal concentrations (LC50[2]).

Laboratory test procedures used in conducting the topsmelt test were very similar to those of the mysid tests. Topsmelt larvae (15 days old at the time of test initiation) were obtained from Aquatic Biosystems, Inc. Larvae were exposed for 48 hours in a static test to five dilutions of ballast water samples: 6.25, 12.5, 25, 50, and 100 percent ballast water and to a dilution water control. Five replicate 1-L test chambers, each containing 500 ml of test solution and 5-8 fish, were used at each treatment level in all tests. Organisms were maintained at a water temperature of 25 ± 1°C under a 16:8 hour light:dark cycle. Test solutions were not aerated during the test and larvae were not fed. Organisms were monitored for survival/mortality daily over the course of the test and at the end of the test. The results were used to determine median lethal concentrations (LC50s).

6.6 Bacteria

6.6.1 Culturable Heterotrophic Plate Count

The number of viable heterotrophic bacteria were determined by performing a culture-based microbiological procedure. During the shipboard experiment, ballast water was collected from the ozonated ballast tank and the control ballast tank in 5-L Niskin oceanographic bottles. For enumeration of the microorganisms, a sample from the Niskin bottle was placed in a 1-L sterilized Nalgene bottle. These bottles were placed on ice in a cooler on board the ship, transported to the University of Washington laboratory on ice, and maintained on ice until the samples were processed in the laboratory. Samples were processed at the University of Washington in the Herwig laboratory within 24 hours of collection on board the *S/T Tonsina*. The numbers of culturable heterotrophic bacteria were determined on Marine R2A Agar (Section 6.6.2) by using two methods. Aliquots of ballast water were inoculated onto the surface of the agar by using the spread plate method or a larger volume of seawater was filtered through a membrane filter (Gelman Metricel Black 47-mm diameter, 0.45-µm pore size filters). Filters were placed on the surface of Marine R2A Agar contained in a 50-mm diameter petri plate. Filters were rolled onto the agar surface to prevent air bubbles from forming between the filter and agar. Larger 100-mm diameter petri dishes were used for samples that were inoculated onto the agar by the spread plate method. Samples were generally inoculated in triplicate for each dilution, except for some filtered samples that were inoculated in duplicate. Inoculated media were incubated at room temperature (approximately 22 °C) in the dark. Bacterial colonies were counted on the spread-plate agar surfaces and membrane filters after 4 days when the colonies were large enough to see but not crowding against one another. The spread plate media were enumerated after 7 days of incubation.

The membrane filtration method was used for the ozonated samples to increase the sensitivity of the assay. A much larger volume of seawater was examined using the membrane filtration method. Filtration was performed with 10 and 100 mL of the sample, and spread plates were inoculated with 100 µL of the original sample or 100 µL from a serial dilution of the

[2] The LC50 represents the concentration of a test material (i.e., ballast water) necessary to kill 50% of a population of exposed organisms

sample. A marine diluent was prepared for the serial dilutions. The formulations for the bacteriological media used in the experiment follows.

6.6.2 Marine R2A Agar

Marine R2A Agar is a modification of a medium that is recommended by EPA for the enumeration of the total number of culturable heterotrophic bacteria in freshwater samples. Marine R2A agar (Table 6.6.1) was supplemented with the salts that are found in seawater. The Herwig Lab has developed a marine salts solution called ONR Seawater Salts (Table 6.6.2, 6.6.3) that contains the major cations and anions found in seawater. For Marine R2A agar, the contents of ONR Seawater Salts replaces distilled water, the liquid that is used to prepare R2A agar. The ONR Seawater Salts solution was prepared as a 10X solution so that 100 mL of the 10X solution is used to prepare 1,000 mL of Marine R2A Agar. The pH of medium was adjusted to 7.6 and the medium was sterilized by autoclaving at 121 °C. Following autoclaving, the medium was cooled in a water bath to 50 °C. ONR Divalent Cations solution (20.0 mL per liter of 50X solution) and ONR FeCl solution (5.0 mL per liter of 200X solution) were added to the liquid. Divalent cations and Fe were added to the medium after autoclaving to minimize the formation of a precipitate in the medium. The dehydrated form of R2A agar medium is commercially available from Difco (Detroit, MI).

Table 6.6.1. R2A Agar (Difco) constituents.

Yeast Extract	0.5 g
Proteose peptone No.3 or polypeptone	0.5 g
Casamino acids	0.5 g
Glucose (Dextrose)	0.5 g
Soluble Starch	0.5 g
K_2HPO_4	0.3 g
$MgSO_4 * 7H_2O$	0.05 g
Sodium Pyruvate	0.3 g
Agar	15.0 g
Distilled water (ONR Seawater Salts used for Marine R2A)	1,000 ml

Table 6.6.2. ONR Seawater Salts solutions. (1X Concentrations in final Marine R2A Agar preparations)

10X Salts	(g/L)	50X Divalent Cation Salts	(g/L)	200X Fe Salts	(g/L)
NaCl	227.916	$MgCl_2 * 6H_2O$	55.908	$FeCl * 4H_2O$	0.40
Na_2SO_4	39.771	$CaCl_2 * 2H_2O$	7.277		
KCl	7.232	$SrCl_2 * 6H_2O$	0.121		
NaBr	0.833				
$NaHCO_3$	0.309				
H_3BO_3	0.266				
NaF	0.026				

Table 6.6.3. Marine salts, final concentration in R2A Agar	
Salt	**Per Liter (g)**
NaCl	22.792
$MgCl_2 * 6H_2O$	11.182
Na_2SO_4	3.977
$CaCl_2 * 2H_2O$	1.455
KCl	0.723
NaBr	0.083
$NaHCO_3$	0.0309
H_3BO_3	0.0266
$SrCl_2 * 6H_2O$	0.024
NaF	0.0026
$FeCl_2 * 4H_2O$	0.002

Marine Mineral Salts Diluent. ONR Seawater Salts solution with no added carbon source. Dilution blanks containing 9.0 ml were dispensed into 16 x 150 mm screw cap tubes. Autoclave for 15 min at 121 °C. Final pH 7.6.

6.6.3 Bacterial Regrowth in Ozonated Ballast Water

To examine the ability of heterotrophic microorganisms to regrow in the period following treatment on board the *S/T Tonsina*, samples were collected from the experimental ballast tank and stored for 35 days. Seawater samples were collected at the end of the 5 hours (T3) and 10 hours (T5) of ozone treatment from the A and B columns of the treated tanks. Five liters of seawater were collected and combined from each ozone treatment and placed in a 10-L sterile Nalgene carboy. A 10-L sample was also collected from the untreated ballast tank at the end of the experiment, which provided for a 10-hour (T5) sample. The 10-L seawater samples were placed on ice until they were returned to the Herwig laboratory at the University of Washington. Here, the carboys were placed inside a 10° C incubator and incubated in the dark for 35 days. Samples were removed from the carboys and inoculated onto the heterotrophic medium as described above. The ozonated seawater sample was concentrated by membrane filtration and the untreated seawater sample was inoculated directly onto the surface of the Marine R2A Agar.

6.7 Zooplankton

A 0.3-m diameter 73-μm mesh zooplankton net was used to collect animals to estimate abundance and condition (mortality or moribund). The net was lowered from two openings in the top of both the control and treatment tank to within 0.25 m of the tank bottom and slowly retrieved to the surface. Three replicate zooplankton vertical hauls were taken from each opening before ozone treatment, and after five hours (all experiments) and ten hours (November experiments) of ozone treatment. Samples were gently washed from the net collecting bucket into a new plastic specimen jar and placed on top of a layer of ice in a bucket. For ozone and control treatments, the samples were immediately examined under a dissecting microscope. A field of view at 25x magnification was examined. Animal activity was scored as follows: if animals were moving of their own accord or moved away when probed with a fine needle (a 000 size insect pin mounted on a wooden stick), they were scored as "alive;" if they were not mobile,

but exhibited internal or external movement, they were scored as "moribund;" and if they showed no life, they were scored as "dead." Successive fields of view were examined until a total of 100 organisms were examined. In addition to these counts, qualitative observations were made about which, if any, taxa appeared to be more or less affected by the treatment.

For the exchange experiments, samples were collected and immediately preserved to quantify abundance of zooplankton found in the exchange and control tanks. Changes in abundance were used to estimate the efficacy of exchange. Samples collected from the ozone tanks were preserved for analyses following an assessment of condition.

6.8 Phytoplankton

Dr. Richard Lacouture, Academy of Natural Sciences Environmental Research Center, using the following approach, analyzed all samples collected for phytoplankton analyses. Using a sub-sample from each of the samples, the number of cells present for each species (or lowest taxonomic unit) was counted directly under a compound microscope. First, 200 individual cells were counted for each of 20 files at 500x magnification. This provided data for the number of cells for small species (e.g., microflagellates and dinoflagellates). Second, 20 fields were also examined at 312x magnification, to estimate the number of larger, rarer, forms.

To measure the effect of ozone treatment, changes in concentration (before and after treatment) in the experimental ozone treatment and control tanks were compared. For this comparison, the counts were pooled across taxa to obtain total concentrations of three major groups: dinoflagellates, microflagellates, and diatoms. Although species-level information is also available, the effects on the level of taxonomic group were compared, because the species composition will vary across replicate experiments (i.e., community composition varies in space and time). Thus, this approach allows us to treat each experimental run as a replicate measure and to test for overall effects of ozone treatment across replicates. In contrast, since the community composition will differ among experimental runs, it may not be possible to compare performance at a lower taxonomic level across replicate experiments. Furthermore, this approach (using major taxonomic groups) is similar to the analyses for zooplankton and microbial components of the study.

The species-specific counts also are being used to measure the effect(s) of exchange, whereby changes in the concentration of abundant coastal forms are compared between the control tank and a third, experimental exchange tank (Section 6.4).

Since stains were not used to determine viability, the data collected in these initial experiments measured changes in the number of cells present for each group. Although this measure can easily detect significant mortality and degradation of dinoflagellates and microflagellates, it is much less informative about diatoms, for which silica cells walls (termed "frustules") can remain intact and blur the distinction between live and dead cells. Thus, this approach provides a good coarse measure only for the first two groups and not diatoms.

6.9 Laboratory Ozone Toxicity Tests

The effects of ozone were tested on marine vertebrates and invertebrates including juvenile sheepshead minnows (*Cyprinodon variegatus*), larval topsmelt (*Atherinops affinis*),

adult mysid shrimp (*Americamysis bahia*), and adults of two amphipod species (*Leptocheirus plumulosus*, and *Rhepoxinius abronius*). All organisms except *R. abronius* were received in good condition from Aquatic Biosystems, Inc. Transport water chemistry was measured upon arrival at ENSR. The range of temperatures was 18-22 °C, the range of pH was 7.9-8.5 and the range of salinity was 24-32 ‰. *R. abronius* were collected in the field near Anacortes, WA, shipped overnight, and received in good condition from P. Dinnel at University of Western Washington, Anacortes. Transport water was analyzed upon arrival (11 °C, pH 6.8, 31 ‰).

On the day prior to testing, 2.5-gallon or 5-gallon glass aquaria were placed in a 21-25 °C water bath and filled with reconstituted waters produced by adding Forty Fathoms Crystal Sea (Marine Enterprises International, Baltimore, MD) to Milli-Q water. Salinity values of reconstituted waters ranged from 28-32 ‰. For testing of *R. abronius*, the water bath was set at 15 °C. The water bath was covered with a frame with a plexiglass top and plastic flaps on the sides and aquaria were left overnight for equilibration of temperature. Salinity values in aquaria on testing days ranged from 29-31 ‰.

Ozone was dispensed using a Nutech 03, Inc. (McLean, VA), Model SC-10 ozone generator. Total flow through the system was 2500 mL/minutes. Flow to each tank was controlled with a flow meter (Gilmont Instruments, Tube Number NO12-10 with glass float). Nominal flow rates for experiments where 5-gallon tanks were used (*C. variegatus, R. abronius, A. affinis*) were 40, 30, 20, and 10, which corresponded to 97.5, 63.2, 38.6, and 20 mL/minutes of ozone gas. These corresponded to ozone loading rates of 0.43, 0.28, 0.17, and 0.09 mg O_3/L/minutes. The controls received compressed, ambient air at 97.5 mL/minutes (nominal flow rate of 40). Nominal flow rates for experiments where 2.5-gallon tanks were used (*A. bahia, L. plumulosus*) were 20, 15, 10, and 5, which corresponded to 38.6, 28.3, 20, and 13.1 mL/minutes of ozone gas. These corresponded to ozone loading rates of 0.34, 0.25, 0.17, and 0.11 mg O_3/L/minutes. The controls received compressed, ambient air at 38.6 ml/minutes (nominal flow rate of 20). The smaller tanks were used to facilitate counts of organisms.

Each test included a total of five chambers, with one chamber tested per treatment. Ten organisms were placed in each chamber. Small pieces of nylon mesh (five pieces, approximately 2 in. x 2 in.) were also placed in each chamber for *R. abronius* and *L. plumulosus* as substrate. Before the initiation of ozone treatment, temperature, dissolved oxygen (DO), and pH were measured for each chamber. Total residual oxidants and ORP were measured in each chamber for up to five hours at approximately 0, 0.5, 1, 2, 3, 4, and 5 hours. Total residual oxidants were reported as total residual chlorine (TRC) as measured by a Hach Pocket Colorimeter using a DPD/KI method (APHA 1998). This procedure was equivalent to the U.S.E.P.A. methods 330.5 for wastewater and standard method 4500-Cl G for drinking water. Oxidation-reduction potential was measured by using an Orion 290A meter with a Cole-Palmer Combination ORP probe (Pt electrode, Ag/AgCl reference cell). Experiments were terminated within the five-hour period if organisms in all treatments receiving ozone were moribund. Counts of survivors were conducted at the same time as chemistry measurements were made. In exposures of *A. affinis* and *R. abronius*, DO was measured at 2 hours and 4 hours, if the test was not previously terminated.

A preliminary experiment, testing for the effects of Mud-Out® Marine Mud Remover (Northeast Technical Services Co., Olmsted Falls, OH) on TRO and ORP measurements, was conducted using two 10-gallon tanks and laboratory-produced seawater. Flows to both tanks were 97.5 mL/minutes corresponding to an ozone-loading rate of 0.43 mg O_3/L/minutes. At the initiation of ozone treatment, temperature, DO, and pH were measured in each chamber. Salinity was 32 ‰. Total residual oxidants and ORP were measured at 0.33, 0.66, 1, 2, and 3 hours.

Data were analyzed for LC50 or EC50 values using the Trimmed Spearman-Karber test. An overall LC50 or EC50 was calculated for each species using 9-10 ORP concentrations recorded across all ozone flows during the duration of the exposure.

Post-exposure recovery test. Biological effects of the exposure of organisms to ozonated water were examined using 10-day old *A. bahia.* Organisms were received in good condition from Aquatic Biosystems, Inc.. Transport water chemistry was measured upon arrival at ENSR. Water temperature was 23 °C, pH was 7.9 and salinity was 28 ‰. Organisms were placed at 19 °C for 2 hours to acclimate to temperature.

On the day prior to testing, 5-gallon glass aquaria were placed in a 19 ± 2 °C water bath. Each aquarium was filled with 16 L of reconstituted seawater (Forty Fathoms Crystal Sea, Marine Enterprises International, Baltimore, MD, reconstituted in Milli-Q water). Salinity of the water was 29 ‰. The water bath was covered with a frame with a plexiglass top and plastic flaps on the sides and aquaria were left overnight for equilibration of temperature. Salinity values in aquaria on testing days were 29 ‰.

Ozone was dispensed using a Nutech 03, Inc., Model SC-10 ozone generator as described previously. Ozone flow rates were 97.5, 63.2, 38.6, and 20 mL/minute. These corresponded to ozone loading rates of 0.43, 0.28, 0.17, and 0.09 mg O_3/L/minute. The control received compressed, ambient air at 97.5 mL/minute.

Each test included five chambers with one chamber tested per treatment. Ten organisms were placed in each chamber. At the initiation of ozone treatment, temperature, DO, pH, TRO, and ORP were measured in each chamber. A Hach Pocket Colorimeter and DPD/KI reagent were used to measure TRO, which was reported as mg/L TRC. Oxidation-reduction potential was measured using an Orion 290A meter with a Cole-Palmer Combination ORP probe (Pt electrode, Ag/AgCl reference cell). Measurements of ORP and TRO were again made at approximately 75 minutes. At 90 minutes, the test was terminated and survivors were siphoned out of the tanks. They were placed in 250-ml beakers with 200 ml of clean seawater (29 ‰). Organisms were fed *Artemia franciscana* (100 µL/beaker) and were placed in a 19 ± 2 °C water bath. After 24 hours, organisms were checked for mortality or moribund conditions. Dead organisms were removed. Organisms were fed *A. franciscana* and were placed back in a 19 ± 2 °C water bath. After 48 hours, organisms were again checked for mortality or movement. The experiment was then terminated.

Latent toxicity test. The effects of ozonated water on 8-day old *A. bahia* were examined immediately after ozonation, 24 hours after ozonation, and 48 hours after ozonation. *A. bahia* were received in good condition from Aquatic Biosystems, Inc. on three consecutive days.

Transport water chemistry was measured upon arrival at ENSR. Water temperature was 24 °C, pH was 7.6, and salinity ranged from 25-28 ‰. Organisms were removed to a beaker and placed in a 20 °C chamber to acclimate to temperature. They remained in the chamber for 2 hours.

A 5-gallon glass aquarium was filled with approximately 16 L of reconstituted water (Forty Fathoms Crystal Sea in Milli-Q water) and placed in a 19 ± 2 °C water bath. Salinity of the water was 28 ‰. The water bath was covered with a frame with a plexiglass top and plastic flaps on the sides and the aquarium was left to equilibrate to 19 °C. After equilibration, ozone was dispensed as described previously. The ozone flow rate to the single aquarium was 97.5 ml/minute, corresponding to an ozone-loading rate of 0.43 mg O_3/L/minute. Total residual oxidants and ORP were measured at 1 hour and 1.5 hours. Targeted TRO and ORP values for the latent toxicity test were greater than 4.0 mg/L and greater than 700 mV. At 1 hour, TRO and ORP were 3.34 mg/L and 744 mV. At 1.5 hours, values were 5.20 mg/L and 755 mV; ozonation thus was terminated at 1.5 hours. All 16 L were removed from the aquarium into a 20-L low-density polyethylene Cubitainer (Hedwin Corporation, Laporte, IN).

Exposure concentrations were mixed using ozonated water and reconstituted seawater. Percentage mixtures were 100 (ozonated water only), 75, 50, 25, and 0 (seawater only). For each mixture, three 500-ml glass beakers containing 300 mL each were prepared. A portion of each mixture was set aside for chemistry measurements. Temperature, pH, DO, conductivity, salinity, ORP, and TRO were measured for each. *A. bahia* were added at 10 organisms/beaker. Mysids were fed *A. franciscana* at 0.2 ml/beaker at the time of test initiation. Beakers were placed in a 19 ± 2 °C water bath and loosely covered with plexiglass. After 24 hours, counts of mortalities were conducted. Dead organisms were removed from beakers. Water samples from each set of three replicates were composited. Temperature, pH, DO, conductivity, salinity, ORP, and TRO were measured. Mysids were fed *A. franciscana* at 0.1 mL/beaker. Beakers were returned to the 19 ± 2 °C water bath and loosely covered. After 48 hours, dead organisms were again counted and water chemistry parameters were measured. The test was then terminated.

The procedure described above was conducted for water collected immediately after ozonation into the 20-L Cubitainer (0 hours), water collected and then held 24 hours in the same 20-L Cubitainer (24 hours), and water collected and then held 48 hours in the same 20-L Cubitainer (48 hours). Each test required 2.5 L of ozonated seawater. Ozonated water was stored in the Cubitainer at 12 °C with no headspace. Stored ozonated water was warmed to 19 °C before mixing and adding to the 24 hours and 48 hours tests. Seawater was at 20 ± 2 °C before mixing.

Data were analyzed for L50 values using the Trimmed Spearman-Karber test. Values were calculated as a function of % ozonated water. ORP and TRO values measured immediately after mixing. The mortality data used were those collected at 48 hours.

Tubing study. A test was conducted to determine if the tubing placed in ballast tanks to collect water samples in future experiments might affect TRO or ORP measurements. A 5-gallon glass aquarium was filled with approximately 16 L of reconstituted water (Forty Fathoms Crystal Sea in Milli-Q water) and placed in a 19 ± 2 °C water bath. Salinity of the water was 28 ‰. The water bath was covered with a frame with a plexiglass top and plastic flaps on the sides

and the aquarium was left to equilibrate to 19 °C. After equilibration, ORP and TRO were measured. Then ozone was dispensed as described previously. The ozone flow rate to the single aquarium was 97.5 ml/minute, corresponding to an ozone-loading rate of 0.43 mg O_3/l/minute. Oxidation-reduction potential was measured at 1 hour to determine if the value was greater than 700 mV.

A 100-foot section of 3/8 x 0.62 inch polyethylene tubing (US Plastic Corporation, Lima, OH) was flushed with 12 L of Milli-Q water followed by 12 L of seawater. Sampling began after the ORP measurement was taken at 1 hour. Three samples were taken from the upper third of the water column. Then, this section was siphoned and three samples were taken at the end of the siphon. ORP and TRO were measured in all six samples. This procedure was repeated for the middle third and bottom third of the water column. All samples were collected and tested between 1 hour and 2 hours. Ozone production was halted at 2 hours.

TRO and ORP measurements of samples from the water column were compared by calculating the statistic called the Students t-test to TRO and ORP of samples collected after siphoning for the upper, middle and lower third of the aquarium. Differences were considered significant at $p < 0.05$.

6.10 Ozone Experiment

Sampling Locations and Frequency. Given that the maximum time available for ballast tank ozonation during a typical voyage from Port Angeles to Valdez, AK is 3.5 days, it has been estimated that each ballast tank may only be ozonated for a maximum of 5 hours. This estimate assumes: 1) all the ballast tanks are full; 2) all tanks are going to be discharged into coastal waters; and thus 3) all ballast water requires treatment. Longer ozonation periods may also be possible to achieve, so testing entailed both a single 5-hour exposure to provide a conservative evaluation of ozonation effectiveness at this minimum exposure time (experiment 1 in September 2001), as well as two 10-hour exposures to evaluate effectiveness during longer exposure times (experiments 2 and 3 in November 2001).

Sampling locations and frequency depended on the type of data being collected and varied somewhat between the three experiments. For simplicity in this initial study, reliance was placed primarily upon grab samples and vertical net tows collected from several vertical access points (manways or Butterworth® openings) in the treatment and control tanks. All samples for water chemistry and microbiota were collected at the beginning and end of the ozonation period, as well as at 2.5-hour increments to evaluate effectiveness at intermediate times. Larger organisms (e.g., plankton tows) were collected from two different vertical Butterworth® hatches in each tank at the 0 hours and 5 hours time points, as well as at the 10 hours time points during the 10-hour experiments (Figure 6.1.1). Caged organisms were also placed at four depths below two or three of the vertical access points. Finally, composite samples were collected from several discrete Niskin samples for use in WET testing.

Chemistry and microbiology sampling. Samples were collected from each tank using Niskin water bottles vertically deployed through two Butterworth® openings (Figure 6.1.1). Samples were collected from three different depths in the vertical side tanks: 10 feet below the

surface of the water (depth 1); 30 feet below the surface (depth 2); and 50 feet below the surface (depth 3). Total water column depth varied between tanks and experiments, but averaged about 65 feet.

Plankton sampling. Plankton tows were obtained from two vertical openings over each ballast tank (except for the two 10-hour experiments, where the control tank was sampled through only one access port). Triplicate tows were taken immediately prior to ozonation, after five hours of ozonation, and also, during the 10-hour experiments, after ten hours of ozonation.

Caged organism sampling. The abundance and taxonomic composition of the ballast water organisms collected during tank fill cannot be predicted or repeated, especially for large mobile organisms. Therefore, caged organisms were introduced to the treatment and control tanks during the ozone experiments to provide known and repeatable biotic assemblages against which ozonation effectiveness could be tested for this group. Caged organisms included mysids, amphipods, shore crabs, and sheepshead. Each was suspended from manhole access ports prior to the start of ozonation for retrieval after completion of ozonation.

Overall sampling frequency. Sampling frequency depended on sample type (Tables 6.10.1 and 6.10.2). More sampling effort was concentrated at the beginning and end of the study, but some chemical and biological data were collected (on a limited basis) mid-way through the experiment. Ozone chemistry and microbes (bacteria counts) were sampled frequently to track changes in ozone chemistry (and its residuals), and biological responses obtained from the same samples. These samples were collected from the deck via Niskin samplers (Table 6.10.1). Samples from plankton tows were collected at the beginning and end (0 hours and 5 hours) of the 5-hour ozone experiment and at the beginning, midpoint and end (0 hours, 5 hours, 10 hours) of the 10-hour ozone experiments (Table 6.10.2). Caged organisms were suspended from Butterworth® openings in the tanks just prior to ozonation, and collected as soon as possible after treatment was completed for the counting of living/moribund/dead organisms. Water samples for WET testing and chemistry splits were also collected using Niskin grabs from both ballast tanks immediately after completion of ozonation.

As shown in Tables 6.10.1 and 6.10.2, sampling times for the control and treatment tanks was staggered to facilitate efficient use of personnel. This eliminated the logistically difficult task of sampling both the control and treatment tanks simultaneously, and ensured that time-sensitive ozone chemistry measurements (e.g., ozone, bromine, ORP) could be taken within minutes of collection. While this offset the experiment initiation in each tank to a minor degree, this should not have substantially impacted the results because the control did not use any kind of aeration in this experiment. Specific personnel assignments, volumes required, and materials needed for Niskin sampling are included in Appendix A.

Table 6.10.1. Description and collection schedule for samples collected via Niskin grabs. Numbers at individual time points denote samples per individual Niskin grab. Totals represent all samples collected from each of two vertical access ports per tank, and collected from each of three different depths.

		Basic Water Quality Parameters							Ozone and Residuals					Biology			Toxicity
Analyte		Salinity	Temp	DO	pH	Nitrite	Nitrate	React. Phos.	Ozone	Bromine	ORP	Bromate	Bromoform	Bacteria	Phytoplankton	Zooplankton	
Analytical Method		Conductivity Meter	pH meter	DO meter	pH meter	Std. Colorimetric	Std. Colorimetric	Std. Colorimetric	Accuvac	Accuvac	probe	ion chrom	GC	direct count	direct count	direct count	WET test
Location Analyzed		on board	on board	on board	on board	lab	lab	lab	on board	on board	on board	lab	lab	lab	lab	on board	lab
Hour	Tank	No. of samples	No. of samples	No. of samples	No. of samples	No. of samples	No. of samples	No. of samples	No. of samples	No. of samples	No. of samples	No. of samples	No. of samples	No. of samples	No. of samples	No. of samples	No. of samples
-1	control	3	3	3	3	3	3	3	6	6	3	3	6	3	3	3	
0	treatment	3	3	3	3	3	3	3	6	6	3	3	6	3	3	3	
1.5	control	3	3	3	3				6	6	3	3	6	3	3	3	
2.5	treatment	3	3	3	3				6	6	3	3	6	3	3	3	
4	control	3	3	3	3				6	6	3	3	6	3	3	3	
5	treatment	3	3	3	3				6	6	3	3	6	3	3	3	
6.5	control	3	3	3	3				6	6	3	3	6	3	3	3	
7.5	treatment	3	3	3	3				6	6	3	3	6	3	3	3	
9	control	3	3	3	3				6	6	3	3	6	3	3	3	
10	treatment	3	3	3	3				6	6	3	3	6	3	3	3	
10.5	treatment																2

Table 6.10.2. Description and schedule for samples to be collected via vertical plankton net tows. Sample numbers at each time period indicate total number of samples to be collected.

Tank		Zooplankton Sps. Composition and Abudnance
Method:		Direct microscope count
Analysis:		Live vs. dead, and preserved?
Location:		On Board, UW
Time:		
-1 Hr	Treatment	2 columns * 3 replicates = 6
0 Hr (begin ozonation)		
1Hr	Control	6
2.5 Hr	Treatment	6
3.5 Hr	Control	6
5 Hr (stop ozone)	Treatment	6
6Hr	Control	6
TOTAL SAMPLES =		24

6.11 Ozone Treatment Control

An experimental control for ozonation could consist either of bubbling ambient air through ozone diffusers at approximately the same rate as ozone-containing gas, or no treatment of any kind. Aeration would mimic the physical disturbance and water column mixing of the ozone treatment, but it may be possible that aeration could also negatively impact some planktonic organisms. Therefore, for this preliminary study, we selected a no-treatment control. However, critically important to the success of this design was that both the ozonated and control tanks were filled at the same time and with the same water mass.

6.12 Caged Organism Studies

In situ caged organism exposures were employed to evaluate the efficacy of ozone ballast water treatment across a range of aquatic organisms. Prior to initiation of ozone treatment, test organisms were placed in cages and suspended via a tether line in both the ozone-treated and control tanks. Organisms remained in the ballast water tanks throughout the 5- or 10-hour ozone exposures after which they were evaluated for survival and morbidity. A variety of vertebrate and invertebrate aquatic organisms were evaluated including: mysid shrimp (*A. bahia*), sheepshead minnows (*C. variegatus*), shore crab (*Hemigrapsus nudus*), and amphipod (*R. abronius)*. These organisms were chosen based on their known sensitivity or hardiness (shore crabs) to a variety of aquatic toxicants and their use as "standard" laboratory test organisms. Organisms were obtained from a commercial supplier in Fort Collins, CO (mysids and sheepshead) or field collected from areas near Anacortes, WA (shore crabs and amphipods). All organisms were acclimated and maintained under either static or flowing seawater conditions at Western Washington University's Shannon Point Marine Laboratory, Anacortes, WA. Prior to testing, organisms were placed in individual exposure chambers and transported to the *S/T Tonsina* in ice chests containing aerated seawater.

Groups of caged organisms were placed into the control and treatment tanks. Each exposure group consisted of a plastic bucket containing sand (Figure 6.12.1) connected to a tether rope by which it could be lowered through the access hatches to the bottom of the ballast water tank. Buckets were used for deployment of amphipod exposure chambers, with chambers for the other three species being suspended from the tether rope at 10, 30 and 50 feet above the bottom (Figure 6.12.2).

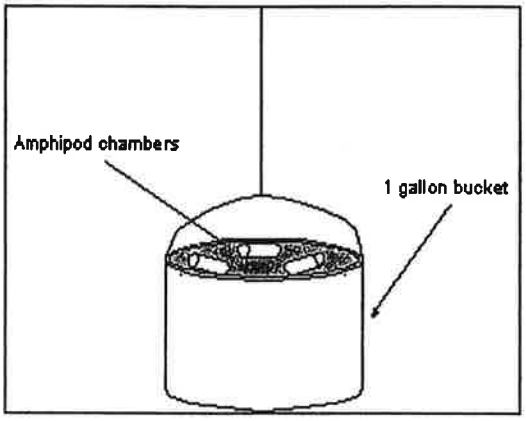

Figure 6.12.1. Close-up of the bucket with replicate amphipod chambers.

For the amphipods, three *in situ* chambers were put into the top half of each bucket (sand in the bottom half acted as anchors) (Figure 6.12.1). Amphipod chambers were modeled after that described by Tucker and Burton (1999), and contained 10 amphipods each (30/bucket). Amphipod chambers were constructed of 5-cm diameter clear plastic tubes approximately 12-cm long capped at each end with polypropylene caps. Each chamber contained two rectangular 3 x 5-cm openings covered with 1-mm polypropylene-woven screen and held in place using silicon glue. All exposure chambers were soaked in both freshwater and seawater for 24 hours each to assure that chamber construction materials or the silicon glue did not impart any toxicity. Amphipod exposure chambers were held in the plastic buckets by means of a coarse mesh polyethylene net placed around each bucket.

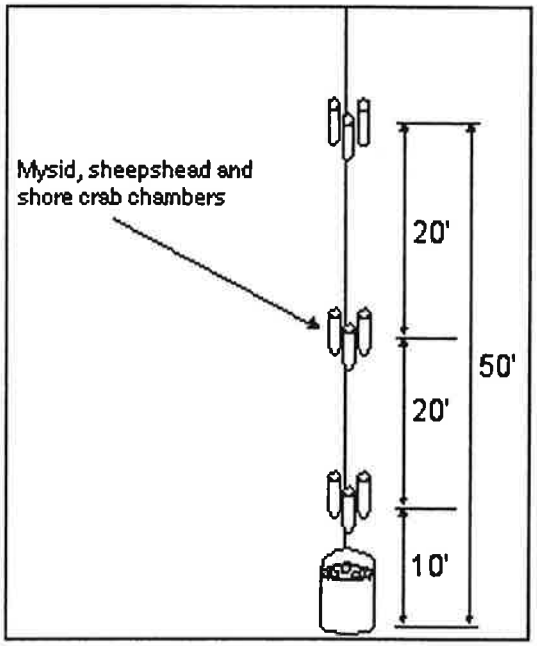

Figure 6.12.2. Drawing of one complete set of exposure chambers.

Exposure chambers for each of the other three test species (i.e., mysid shrimp, sheepshead minnow, and shore crab) were attached to the tether rope at intervals of 10, 30, and 50 feet above the bucket (Figure 6.12.2).

For mysids and sheepshead, ten individual organisms (of a single species) were placed into the clear-plastic exposure chambers, constructed as above and containing two rectangular windows (3 x 5 cm) covered with 750-μm mesh (for mysids) or 1-mm mesh (for sheepshead), one on either side of the chamber, and each chamber was capped with polypropylene caps. For shore crabs, ten individual organisms were placed into commercially available plastic crab bait buckets (11 cm high x 9 cm diameter) drilled with numerous 8 mm holes. Groups of three chambers (one for each species) were placed in coarse-mesh polyethylene nets and attached to the tether rope via clamps.

At the completion of the 5- or 10-hours ozone treatment periods, cages were removed and the number of surviving organisms recorded immediately. Additionally, numbers of animals appearing moribund (or failing to rebury in sand for the amphipods) were also recorded. Results are reported as percent survivorship and percent moribund for each taxon.

7 RESULTS AND DISCUSSION

7.1 Ozone Delivery

Table 7.1.1 summarizes the water volume capacity of both sections of the ozone treatment tank (No. 3 port ballast tank) and number of ozone diffusers in each section, as well as the calculated ozone loading rate in each section for each of the three experiments. Note that the "vertical/sample portion 3P" row shows the information pertaining to the vertical wing tank that is the portion from which samples for these experiments were taken. The ozone loading rate in this wing tank increased by 22 % between experiments 1 and 2, and then by 87.5 % between experiments 2 and 3. This increase in ozone loading is generally reflected in the biological and chemical data presented below. The low number of diffusers in this wing tank is notable, given the variability of effectiveness within the tank that the biological data reveal.

7.2 General Chemical Characteristics

Several general water quality parameters were recorded during the three experiments. These water quality parameters were dissolved oxygen (Table 7.2.1), pH (Table 7.2.2), salinity (Table 7.2.3) temperature, (Table 7.2.4), dissolved organic carbon (Table 7.2.5), phosphate ion (Table 7.2.6), silica (Table 7.2.7), nitrate ion (Table 7.2.8), nitrite ion (Table 7.2.9) and ammonia (Table 7.2.10).

Dissolved oxygen is generally considered an important parameter in water quality to sustain aerobic life. In general, highly oxygenated water is considered "healthy" for biota. Because oxygen is the stable product of ozone, it was measured to determine whether its concentration would increase with ozonation.

Another generally important water quality parameter is pH. This provides a measure of the acid/base equilibrium in water. In the case of ozonation, it also is important in determining the distribution of the reaction by-product of ozone and bromide ion, bromine.

Salinity is the measure of the total amount of "salts" in water. The measure of salinity provides an estimate of the amount of oceanic water in the ballast water. Of interest in the ozonation of seawater are the reactions of ozone with bromide ion. It was thought that no change in salinity would occur during ozonation.

The organic fraction of the water (DOC) is of interest because: 1) it provides an indication of the quality of the water being used for ballast purposes (the lower the DOC the higher the water quality), and, 2) the reaction of bromine with DOC leads to the formation of bromoform, which is one of the ozone reaction by-products of potential interest.

The nutrient data are indicative of general ballast water quality, and the potential of this water to support the growth of phytoplankton and other microorganisms.

Table 7.1.1 Ozone production, distribution and loading in the treatment tank.

				diffuser density	O3 production			O3 distributition			O3 loading rate		
	volume	volume	No. of O3	(BBLS per	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3	Exp 1	Exp 2	Exp 3
Ballast Tank	(BBLS)	(L)	diffusers	diffuser)	gram/hr	gram/hr	gram/hr	%	%	%	(mg/l/hr)	(mg/l/hr)	(mg/l/hr
3P	19,608	3,117,084	72	272	1460	1760	1660				0.47	0.56	0.53
horizontal portion 3P	11,802	1,876,164	56	211				50%	40%	0%	0.39	0.38	0.00
vertical/sample portion 3P	7,723	1,227,725	16	483				50%	60%	100%	0.59	0.72	1.35
average Tonsina tank	*17,925*	2,849,537						*100%*	*100%*	*100%*	*0.51*	*0.62*	*0.58*

Table 7.2.1. Summary of the results of dissolved oxygen (DO) in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).				
Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		DO (mg/L as O_2)		
A10- Treatment	T-0.0	Not Sampled	9.0	6.6
	T-2.5	Not Sampled	10.3	13.8
	T-5.0	Not Sampled	14.3	17.5
	T-7.5	Not Sampled	Not Sampled[1]	19.1
	T-10.0	Not Sampled	21.8	20.2
A30- Treatment	T-0.0	Not Sampled	9.2	6.8
	T-2.5	Not Sampled	10.5	12.7
	T-5.0	Not Sampled	14.5	19.2
	T-7.5	Not Sampled	Not Sampled	19.2
	T-10.0	Not Sampled	21.5	19.2
A50- Treatment	T-0.0	Not Sampled	8	7.8
	T-2.5	Not Sampled	7.9	8.3
	T-5.0	Not Sampled	15.3	13.1
	T-7.5	Not Sampled	Not Sampled	16.5
	T-10.0	Not Sampled	14.2	18.1
B10- Treatment	T-0.0	Not Sampled	8.9	6.1
	T-2.5	Not Sampled	8.4	7.8
	T-5.0	Not Sampled	11.9	15
	T-7.5	Not Sampled	Not Sampled	17.4
	T-10.0	Not Sampled	14.9	19.6
B30- Treatment	T-0.0	Not Sampled	9.3	6.3
	T-2.5	Not Sampled	8.6	8.6
	T-5.0	Not Sampled	11.5	16.8
	T-7.5	Not Sampled	Not Sampled	17
	T-10.0	Not Sampled	18.8	18.8
B50- Treatment	T-0.0	Not Sampled	9.3	6.9
	T-2.5	Not Sampled	7.8	8.8
	T-5.0	Not Sampled	10.6	15.2
	T-7.5	Not Sampled	Not Sampled	16.3
	T-10.0	Not Sampled	19	18.2
C10-Control	T-0.0	Not Sampled	5.9	6.9
	T-2.5	Not Sampled	6.5	7.1
	T-5.0	Not Sampled	6.6	7.4
	T-7.5	Not Sampled	Not Sampled	8
	T-10.0	Not Sampled	8	6.4
C30-Control	T-0.0	Not Sampled	5.8	6.2
	T-2.5	Not Sampled	6.5	6.2
	T-5.0	Not Sampled	6.1	7.9
	T-7.5	Not Sampled	Not Sampled	8.2
	T-10.0	Not Sampled	7.7	6.4

Table 7.2.1. Summary of the results of dissolved oxygen (DO) in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).				
C50-Control	T-0.0	Not Sampled	5.7	5.9
	T-2.5	Not Sampled	6.3	6.9
	T-5.0	Not Sampled	6.4	8.2
	T-7.5	Not Sampled	Not Sampled	7.3
	T-10.0	Not Sampled	6.9	7.4
D10-Control	T-0.0	Not Sampled	Not Sampled	Not Sampled
	T-2.5	Not Sampled	Not Sampled	Not Sampled
	T-5.0	Not Sampled	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D30-Control	T-0.0	Not Sampled	Not Sampled	Not Sampled
	T-2.5	Not Sampled	Not Sampled	Not Sampled
	T-5.0	Not Sampled	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D50-Control	T-0.0	Not Sampled	Not Sampled	Not Sampled
	T-2.5	Not Sampled	Not Sampled	Not Sampled
	T-5.0	Not Sampled	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
[1] During the second experiment, the fourth time interval there was apparently a problem with the DO meter and all of the samples were lost.				

For the two experiments where DO was measured, Experiment 2 and 3, a steady increase was observed in the ballast tanks that were ozonated (Table 7.2.1). The T1 samples for Experiment 2 averaged 8.95 mg/L of O_2 whereas the T1 samples for experiment three averaged 6.75. In both experiments the O_2 concentration increased by at least two-fold during the ozonation period and showed a steady increase with increased ozonation time. This is consistent with ozone decomposition into O_2. The control tank that was sampled showed no consistent pattern of O_2 change.

This increase in O_2 concentration would have a positive effect on water quality for disposal, or dumping in the receiving port of call. An interesting question would be to determine how long the elevated concentration of O_2 remained in the ballast water in closed tanks. It is possible that this dramatic increase in O_2 concentration might have an adverse affect on some organisms entrained in the water at the time of filling. In particular any anaerobic bacteria or organisms that have a low threshold for elevated O_2 concentrations would presumably not survive in this environment for very long.

Table 7.2.2. Summary of the results of pH measurements in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).				
Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		pH		
A10- Treatment	T-0.0	7.5 – 8.0	7.4	7.54
	T-2.5	7.0	7.5	7.7
	T-5.0	7.0	7.5	7.7
	T-7.5	Not Sampled	7.8	7.8
	T-10.0	Not Sampled	7.9	7.8
A30- Treatment	T-0.0	7.0 – 7.5	7.42	7.55
	T-2.5	7.0 – 7.5	7.51	7.86
	T-5.0	7.0 – 7.5	7.55	7.72
	T-7.5	Not Sampled	7.83	7.77
	T-10.0	Not Sampled	7.95	7.82
A50- Treatment	T-0.0	7.0 – 7.5	7.43	7.54
	T-2.5	7.0	7.5	7.6
	T-5.0	7.0 – 7.5	7.54	7.83
	T-7.5	Not Sampled	7.7	7.7
	T-10.0	Not Sampled	7.9	7.89
B10- Treatment	T-0.0	7.5 – 8.0	7.44	7.53
	T-2.5	7.0	7.5	7.6
	T-5.0	7.0 – 7.5	7.49	7.7
	T-7.5	Not Sampled	7.7	7.7
	T-10.0	Not Sampled	7.89	7.79
B30- Treatment	T-0.0	7.0 – 7.5	7.39	7.54
	T-2.5	7.5	7.5	7.6
	T-5.0	7.0 – 7.5	7.49	7.72
	T-7.5	Not Sampled	7.8	7.8
	T-10.0	Not Sampled	7.91	7.83
B50- Treatment	T-0.0	7.0 – 7.5	7.45	7.54
	T-2.5	7.0	7.5	8.0
	T-5.0	7.0	7.5	7.7
	T-7.5	Not Sampled	7.7	7.8
	T-10.0	Not Sampled	7.9	7.8
C10-Control	T-0.0	7.5	7.32	7.97
	T-2.5	7.0 – 7.5	7.46	7.78
	T-5.0	7.5	7.47	7.61
	T-7.5	Not Sampled	7.7	7.64
	T-10.0	Not Sampled	7.74	7.01
C30-Control	T-0.0	7.0 – 7.5	7.46	7.75
	T-2.5	7.0	7.5	7.9
	T-5.0	7.0 – 7.5	7.44	7.63
	T-7.5	Not Sampled	7.7	7.6
	T-10.0	Not Sampled	7.71	7.7

Table 7.2.2. Summary of the results of pH measurements in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).				
Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		pH		
C50-Control	T-0.0	7.0 – 7.5	7.45	7.76
	T-2.5	7.0	7.5	7.7
	T-5.0	7.0 – 7.5	7.46	7.84
	T-7.5	Not Sampled	7.7	7.7
	T-10.0	Not Sampled	7.73	7.67
D10-Control	T-0.0	7.0 – 7.5	Not Sampled	Not Sampled
	T-2.5	7.0 – 7.5	Not Sampled	Not Sampled
	T-5.0	7.0 – 7.5	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D30-Control	T-0.0	7.0 – 7.5	Not Sampled	Not Sampled
	T-2.5	7.0	Not Sampled	Not Sampled
	T-5.0	7.0 – 7.5	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D50-Control	T-0.0	7.5	Not Sampled	Not Sampled
	T-2.5	7.5	Not Sampled	Not Sampled
	T-5.0	7.0 – 7.5	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled

The pH of the ballast water in the ozonated tanks increased slightly by about 0.3 – 0.4 units during the course of the 10-hour experiments (Experiment 2 and 3) but did not increase in the 5-hour experiment (Experiment 1; Table 7.2.2). pH in the control tanks showed no increase in any of the 5 or 10-hours experiments. These minor fluctuations will probably not have any positive or adverse affects on the chemistry of the ozone, or on the organisms in the ballast tanks.

Table 7.2.3. Summary of the results of salinity measurements in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).				
Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		Salinity (‰)		
A10- Treatment	T-0.0	33.7	35.6	34.1
	T-2.5	33.5	35.3	34.1
	T-5.0	33.6	35.1	33.9
	T-7.5	Not Sampled	35.3	34.0
	T-10.0	Not Sampled	35.2	33.9
A30- Treatment	T-0.0	33.5	35.8	34.1
	T-2.5	33.6	35.3	34.2
	T-5.0	33.5	35.1	34.1
	T-7.5	Not Sampled	35.1	34.2
	T-10.0	Not Sampled	35.1	34.3
A50- Treatment	T-0.0	33.5	35.6	34.1
	T-2.5	33.6	35.3	34.4
	T-5.0	33.4	35.1	34.2
	T-7.5	Not Sampled	35.1	34.3
	T-10.0	Not Sampled	35.1	34.2
B10- Treatment	T-0.0	33.6	35.9	34.2
	T-2.5	33.3	35.4	34.2
	T-5.0	33.5	35.1	34.1
	T-7.5	Not Sampled	35.0	34.1
	T-10.0	Not Sampled	35.1	34.2
B30- Treatment	T-0.0	33.7	35.7	33.9
	T-2.5	33.0	35.4	34.2
	T-5.0	33.5	35.1	34.2
	T-7.5	Not Sampled	35.1	34.2
	T-10.0	Not Sampled	35.1	34.1
B50- Treatment	T-0.0	33.6	35.7	34.3
	T-2.5	33.7	35.3	34.4
	T-5.0	33.4	35.2	34.3
	T-7.5	Not Sampled	35.1	34.2
	T-10.0	Not Sampled	35.1	34.1
C10-Control	T-0.0	33.2	35.4	34.0
	T-2.5	33.5	35.4	34.0
	T-5.0	33.5	35.2	34.0
	T-7.5	Not Sampled	35.0	34.1
	T-10.0	Not Sampled	35.2	33.9
C30-Control	T-0.0	33.5	35.5	34.2
	T-2.5	33.6	35.1	34.3
	T-5.0	33.5	35.3	34.2
	T-7.5	Not Sampled	35.3	34.3
	T-10.0	Not Sampled	35.4	34.1

Table 7.2.3. Summary of the results of salinity measurements in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).				
Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		Salinity (‰)		
C50-Control	T-0.0	33.5	35.2	34.2
	T-2.5	33.6	35.4	34.3
	T-5.0	33.2	35.2	34.2
	T-7.5	Not Sampled	35.4	34.3
	T-10.0	Not Sampled	35.4	34.1
D10-Control	T-0.0	33.5	Not Sampled	Not Sampled
	T-2.5	33.5	Not Sampled	Not Sampled
	T-5.0	33.6	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D30-Control	T-0.0	33.6	Not Sampled	Not Sampled
	T-2.5	33.6	Not Sampled	Not Sampled
	T-5.0	33.5	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D50-Control	T-0.0	33.4	Not Sampled	Not Sampled
	T-2.5	33.5	Not Sampled	Not Sampled
	T-5.0	33.4	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled

Salinity was not affected by the addition of ozone to the ballast water and there was no difference between the treated and untreated (control) tanks in any of the experiments (Table 7.2.3). This is consistent with the decomposition of ozone in saline waters.

Table 7.2.4. Summary of the result of temperature measurements in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).				
Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		Temperature (°C)		
A10- Treatment	T-0.0	15.1	10.3	10.7
	T-2.5	13.8	11.8	10.0
	T-5.0	14.0	11.1	10.6
	T-7.5	Not Sampled	11.2	10.3
	T-10.0	Not Sampled	10.6	9.9
A30- Treatment	T-0.0	14.8	11.1	10.8
	T-2.5	13.5	11.3	11.4
	T-5.0	13.6	11.2	10.3
	T-7.5	Not Sampled	10.5	10.3
	T-10.0	Not Sampled	10.4	10.2
A50- Treatment	T-0.0	14.2	11.9	10.3
	T-2.5	13.3	10.3	10.3

Table 7.2.4. Summary of the result of temperature measurements in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).

Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		Temperature (°C)		
	T-5.0	12.7	10.2	10.4
	T-7.5	Not Sampled	9.4	10.1
	T-10.0	Not Sampled	9.4	10.1
B10- Treatment	T-0.0	14.1	12.0	10.7
	T-2.5	14.1	11.9	9.9
	T-5.0	13.6	11.0	10.1
	T-7.5	Not Sampled	10.6	9.7
	T-10.0	Not Sampled	10.0	9.7
B30- Treatment	T-0.0	15.5	9.8	10.6
	T-2.5	13.2	11.6	10.0
	T-5.0	13.7	11.8	10.1
	T-7.5	Not Sampled	10.4	10.1
	T-10.0	Not Sampled	10.2	9.5
B50- Treatment	T-0.0	15.1	10.2	10.1
	T-2.5	14.1	10.4	10.8
	T-5.0	12.7	11.0	10.7
	T-7.5	Not Sampled	11.1	11.3
	T-10.0	Not Sampled	9.6	10.2
C10-Control	T-0.0	14.4	10.9	10.0
	T-2.5	13.7	10.7	10.0
	T-5.0	13.4	9.9	10.1
	T-7.5	Not Sampled	9.2	9.9
	T-10.0	Not Sampled	9.6	9.6
C30-Control	T-0.0	14.7	10.5	9.9
	T-2.5	13.9	10.6	9.9
	T-5.0	13.4	9.7	10.3
	T-7.5	Not Sampled	9.6	11.4
	T-10.0	Not Sampled	9.5	10.7
C50-Control	T-0.0	14.0	10.6	10.7
	T-2.5	14.6	10.7	10.1
	T-5.0	12.8	10.3	11.1
	T-7.5	Not Sampled	9.8	10.4
	T-10.0	Not Sampled	9.9	10.3
D10-Control	T-0.0	15.7	Not Sampled	Not Sampled
	T-2.5	13.8	Not Sampled	Not Sampled
	T-5.0	13.3	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D30-Control	T-0.0	14.8	Not Sampled	Not Sampled
	T-2.5	13.9	Not Sampled	Not Sampled
	T-5.0	12.8	Not Sampled	Not Sampled

Table 7.2.4. Summary of the result of temperature measurements in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).				
Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		Temperature (°C)		
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D50-Control	T-0.0	13.6	Not Sampled	Not Sampled
	T-2.5	14.5	Not Sampled	Not Sampled
	T-5.0	12.8	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled

The temperature of the ballast water for Experiment 1 was consistently warmer than for Experiments 2 and 3 (Table 7.2.4). This presumably reflects the time of year that the experiments were conducted. During the 5-hour experiment, Experiment 1, the temperature appeared to decrease approximately one degree. During the ten-hour experiments, Experiments 2 and 3, there was no clear trend or change in temperature throughout the study. The minor variations in temperature suggest that this will have neither a positive nor a negative effect on the treatment. However, the lower temperatures may account for lower bromoform formation in Experiments 2 and 3.

Table 7.2.5 summarizes the dissolved organic carbon concentration in the treated and control tanks of the three experiments. These results suggest that the DOC concentration in the ballast water was similar for all of the experiments. Similarly, no trends were apparent as a function of time or ozone treatment in any of the inorganic nutrients (Tables 7.2.6 – 7.2.10).

Table 7.2.5. Summary of the result of dissolved organic carbon (DOC) measurements in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).				
Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		DOC (mg C /L)		
A10 - Treatment	T-0.0	0.95	0.79	1.11
A30- Treatment	T-0.0	0.93	0.85	1.02
A50- Treatment	T-0.0	0.92	0.75	0.98
B10- Treatment	T-0.0	1.01	0.91	1.02
B30- Treatment	T-0.0	0.95	0.92	1.02
B50- Treatment	T-0.0	1.00	0.82	0.84
C10- Control	T-0.0	Not Sampled	0.81	1.08
C30- Control	T-0.0	0.93	0.72	1.18
C50- Control	T-0.0	0.94	0.74	0.81
D10- Control	T-0.0	0.95	Not Sampled	Not Sampled
D30- Control	T-0.0	0.92	Not Sampled	Not Sampled
D50- Control	T-0.0	1.06	Not Sampled	Not Sampled

Table 7.2.6. Summary of the result of ortho-phosphate ion (PO_4^{3-}) measurements in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).

Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		PO_4^{3-} (mg/L)		
A10 - Treatment	T-0.0	0.0635	0.0762	0.0762
A30- Treatment	T-0.0	0.0634	0.0759	0.0748
A50- Treatment	T-0.0	0.0638	0.0758	0.0751
B10- Treatment	T-0.0	0.0633	0.0760	0.0743
B30- Treatment	T-0.0	0.0635	0.0756	0.0746
B50- Treatment	T-0.0	0.0628	0.0763	0.0744
C10- Control	T-0.0	Not Sampled	0.0750	0.0748
C30- Control	T-0.0	0.0638	0.0753	0.0745
C50- Control	T-0.0	0.0639	0.0752	0.0745
D10- Control	T-0.0	0.0623	Not Sampled	Not Sampled
D30- Control	T-0.0	0.0636	Not Sampled	Not Sampled
D50- Control	T-0.0	0.0624	Not Sampled	Not Sampled

Table 7.2.7. Summary of the result of silica, $Si(OH)_4$, measurements in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).

Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		$Si(OH)_4$ (mg/L)		
A10 - Treatment	T-0.0	1.333	1.519	1.508
A30- Treatment	T-0.0	1.330	1.507	1.504
A50- Treatment	T-0.0	1.322	1.494	1.505
B10- Treatment	T-0.0	1.331	1.512	1.516
B30- Treatment	T-0.0	1.325	1.498	1.489
B50- Treatment	T-0.0	1.326	1.497	1.485
C10- Control	T-0.0	Not Sampled	1.510	1.513
C30- Control	T-0.0	1.349	1.506	1.487
C50- Control	T-0.0	1.344	1.497	1.483
D10- Control	T-0.0	1.357	Not Sampled	Not Sampled
D30- Control	T-0.0	1.342	Not Sampled	Not Sampled
D50- Control	T-0.0	1.353	Not Sampled	Not Sampled

Table 7.2.8. Summary of the result of nitrate ion (NO_3^-) measurements in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).

Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		NO_3^- (mg/L)		
A10 - Treatment	T-0.0	0.287	0.399	0.399
A30- Treatment	T-0.0	0.290	0.397	0.401
A50- Treatment	T-0.0	0.290	0.399	0.403
B10- Treatment	T-0.0	0.286	0.398	0.394
B30- Treatment	T-0.0	0.287	0.399	0.399
B50- Treatment	T-0.0	0.289	0.399	0.410
C10- Control	T-0.0	Not Sampled	0.399	0.395
C30- Control	T-0.0	0.294	0.399	0.412
C50- Control	T-0.0	0.294	0.399	0.402
D10- Control	T-0.0	0.289	Not Sampled	Not Sampled
D30- Control	T-0.0	0.291	Not Sampled	Not Sampled
D50- Control	T-0.0	0.291	Not Sampled	Not Sampled

Table 7.2.9. Summary of the result of nitrite ion (NO_2^-) measurements in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).

Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		NO_2^- (mg/L)		
A10 - Treatment	T-0.0	0.004	0.005	0.006
A30- Treatment	T-0.0	0.004	0.005	0.005
A50- Treatment	T-0.0	0.004	0.005	0.005
B10- Treatment	T-0.0	0.004	0.006	0.006
B30- Treatment	T-0.0	0.004	0.005	0.005
B50- Treatment	T-0.0	0.004	0.005	0.005
C10- Control	T-0.0	Not Sampled	0.005	0.004
C30- Control	T-0.0	0.004	0.005	0.006
C50- Control	T-0.0	0.004	0.005	0.005
D10- Control	T-0.0	0.004	Not Sampled	Not Sampled
D30- Control	T-0.0	0.004	Not Sampled	Not Sampled
D50- Control	T-0.0	0.004	Not Sampled	Not Sampled

Table 7.2.10. Summary of the result of ammonium ion (NH_3) measurements in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).				
Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		NH_4 (mg/L)		
A10 - Treatment	T-0.0	0.032	0.076	0.076
A30- Treatment	T-0.0	0.029	0.076	0.075
A50- Treatment	T-0.0	0.031	0.076	0.075
B10- Treatment	T-0.0	0.031	0.076	0.074
B30- Treatment	T-0.0	0.029	0.076	0.075
B50- Treatment	T-0.0	0.028	0.076	0.074
C10- Control	T-0.0	Not Sampled	0.075	0.075
C30- Control	T-0.0	0.028	0.075	0.075
C50- Control	T-0.0	0.028	0.075	0.075
D10- Control	T-0.0	0.027	Not Sampled	Not Sampled
D30- Control	T-0.0	0.026	Not Sampled	Not Sampled
D50- Control	T-0.0	0.027	Not Sampled	Not Sampled

7.3 Ozone/Oxidant Chemistry

7.3.1 Ozone and Bromine.

At every sampling point and time both ozone (Table 7.3.1) and bromine, hypobromous acid/hypobromite ion, (Table 7.3.2) were determined in duplicate from the same Niskin sample.

Table 7.3.1. Ozone concentration in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).				
Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		Ozone (mg/L)		
A10- Treatment	T-0.0	0.03, -0.08[1]	0.30, 0.32	0.01, -0.09
	T-2.5	0.56, 0.43	0.19, 0.19	-0.04, -0.01
	T-5.0	0.23, 0.26	-0.06, -0.08	0.03, 0.06
	T-7.5	Not Sampled	Not Sampled	0.09, 0.01
	T-10.0	Not Sampled	0.07, 0.25	0.07, 0.06
A30- Treatment	T-0.0	0.00, 0.00	0.39, 0.33	-0.16,0.12
	T-2.5	0.08, -0.01	0.33, 0.18	-0.05, -0.01
	T-5.0	0.20, 0.03	-0.05, -0.06	0.06, 0.04
	T-7.5	Not Sampled	Not Sampled	-0.03, -0.07
	T-10.0	Not Sampled	0.25, 0.06	0.48, 0.10
A50- Treatment	T-0.0	-0.11, -0.02	0.57, 0.32	-0.09
	T-2.5	0.15, 0.14	0.48, 0.07	-0.03, -0.01
	T-5.0	0.24, 0.26	0.49, -0.06	-0.03, -0.32
	T-7.5	Not Sampled	0.49	0.04, -0.07

Table 7.3.1. Ozone concentration in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).				
Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		Ozone (mg/L)		
	T-10.0	Not Sampled	0.38, 0.32	0.25, -0.04
B10- Treatment	T-0.0	0.05, 0.02	0.46, 0.31	-0.02, -0.05
	T-2.5	0.00, -0.03	0.05, 0.11	-0.08, -0.02
	T-5.0	0.10, 0.01	0.12, 0.03	0.03, -0.11
	T-7.5	Not Sampled	-0.09	-0.02, -0.02
	T-10.0	Not Sampled	0.01, 0.04	-0.02,-0.03
B30- Treatment	T-0.0	0.05, 0.02	0.30, 0.37	0.08, 0.10
	T-2.5	0.03, 0.01	0.22, 0.09	-0.06, -0.01
	T-5.0	0.02, 0.00	0.12, 0.08	-0.04, -0.18
	T-7.5	Not Sampled	-0.03	-0.11, -0.14
	T-10.0	Not Sampled	0.27, 0.01	-0.10, -0.16
B50- Treatment	T-0.0	0.01, 0.03	0.30, 0.37	-0.07, 0.01
	T-2.5	0.01, 0.00	0.35, 0.04	-0.01, -0.10
	T-5.0	0.09, 0.17	-0.04, 0.94	0.14, -0.01
	T-7.5	Not Sampled	0.04	0.00, -0.07
	T-10.0	Not Sampled	0.05, 0.01	-0.05, 0.17
C10-Control	T-0.0	-0.04, 0.03	0.50, 0.43	-0.02, -0.21
	T-2.5	0.00, 0.00	0.19, -0.28	0.00, -0.03
	T-5.0	0.24, 0.00	0.78, 0.02	0.18, -0.28
	T-7.5	Not Sampled	-0.02	-0.11, -0.37
	T-10.0	Not Sampled	-0.11	0.15, 0.26
C30-Control	T-0.0	0.18, 0.05	1.36, 0.46	0.03, -0.08
	T-2.5	0.00, 0.00	-0.06, 0.16	-0.05, -0.06
	T-5.0	0.00, 0.00	0.45, 0.08	-0.21, -0.04
	T-7.5	Not Sampled	0.13	-0.19, -0.37
	T-10.0	Not Sampled	Not Sampled	0.00, 0.06
C50-Control	T-0.0	0.11, 0.12	0.48, 0.52	0.09, 0.01
	T-2.5	0.02, -0.02	0.08, 0.04	0.00, 0.01
	T-5.0	0.00, 0.00	0.04, -0.09	-0.07, 0.02
	T-7.5	Not Sampled	0.16	-0.41, 0.12
	T-10.0	Not Sampled	Not Sampled	0.02, -0.14
D10-Control	T-0.0	0.01, 0.05	Not Sampled	Not Sampled
	T-2.5	0.22, 0.24	Not Sampled	Not Sampled
	T-5.0	-0.12, 0.00	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D30-Control	T-0.0	0.12, -0.02	Not Sampled	Not Sampled
	T-2.5	0.02, 0.02	Not Sampled	Not Sampled
	T-5.0	0.00, 0.16	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled

Table 7.3.1. Ozone concentration in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).				
Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		Ozone (mg/L)		
D50-Control	T-0.0	0.18, -0.02	Not Sampled	Not Sampled
	T-2.5	0.22, 0.00	Not Sampled	Not Sampled
	T-5.0	-0.44, 0.00	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
[1] Duplicate analyses conducted				

As discussed in Section 3.2, the lifetime of ozone in marine waters (that is, waters with high concentrations of bromide ion) is expected to be ~5 seconds. The measurements of ozone appeared to fluctuate considerably around zero. These results suggest that no or little ozone was in the samples at the time of analysis.

Table 7.3.2. TRO (milligrams per liter as Cl_2) concentration in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).				
Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		Bromine (mg/L as Cl_2)		
A10- Treatment	T-0.0	-0.13, -0.14[1]	0.06, 0.07	0.07, 0.01
	T-2.5	0.21, 0.43	2.74, 2.80	4.02, 4.07
	T-5.0	0.23, 0.26	2.39, 2.37	OR, OR
	T-7.5	Not Sampled	OR	OR, OR
	T-10.0	Not Sampled	OR[2], OR	OR,OR
A30- Treatment	T-0.0	0.00, 0.00	0.06, 0.04	0.02, 0.02
	T-2.5	0.08, -0.01	2.70, 2.78	3.62, 3.77
	T-5.0	0.20, 0.03	2.84, 2.15	OR, OR
	T-7.5	Not Sampled	OR, OR	OR, OR
	T-10.0	Not Sampled	OR, OR	OR, OR
A50- Treatment	T-0.0	-0.11, -0.02	0.06, 0.05	-0.09, 0.01
	T-2.5	0.15, 0.14	0.37, 0.39	0.32, 0.31
	T-5.0	0.24, 0.26	2.42, 2.39	2.68, 2.72
	T-7.5	Not Sampled	4.70, 4.62	4.53, 4.80
	T-10.0	Not Sampled	OR, OR	OR, OR
B10- Treatment	T-0.0	-0.08, 0.01	0.02, 0.00	-0.04, 0.00
	T-2.5	0.00, -0.03	0.57, 0.56	0.70, 0.59
	T-5.0	0.10, 0.01	OR, OR	2.90, 3.80
	T-7.5	Not Sampled	3.89, 3.94	4.83, 4.72
	T-10.0	Not Sampled	OR, OR	OR, OR
B30- Treatment	T-0.0	0.05, 0.02	0.01, -0.05	-0.01, 0.01
	T-2.5	0.03, 0.01	0.85, 0.84	1.00, 1.08
	T-5.0	0.02, 0.00	OR, OR	3.98, 3.96
	T-7.5	Not Sampled	4.40, 4.37	OR, OR
	T-10.0	Not Sampled	OR, OR	OR, OR
B50- Treatment	T-0.0	0.01, 0.03	-0.03, 0.03	-0.03, -0.04
	T-2.5	0.01, 0.00	0.63, 0.61	0.96, 1.04
	T-5.0	0.09, 0.17	OR, OR	4.14, 4.12
	T-7.5	Not Sampled	3.91, 3.96	OR, OR
	T-10.0	Not Sampled	OR, OR	OR, OR
C10-Control	T-0.0	-0.04, 0.03	-0.29, -0.32	0.00, 0.05
	T-2.5	-0.13, 0.07	0.01, -0.01	0.06, 0.06
	T-5.0	0.24, 0.00	-0.06	0.05, 0.08
	T-7.5	Not Sampled	0.00, -0.04	-0.11, -0.11
	T-10.0	Not Sampled	0.00, -0.01	-0.05, -0.02
C30-Control	T-0.0	0.18, 0.05	-0.39, -0.38	-0.01, -0.05
	T-2.5	0.00, 0.00	0.00, -0.05	0.12, 0.06
	T-5.0	0.00, 0.00	-0.08, -0.10	-0.02, 0.02
	T-7.5	Not Sampled	0.00, -0.06	-0.11, -0.12
	T-10.0	Not Sampled	0.00, 0.00	-0.06, -0.11

Table 7.3.2. TRO (milligrams per liter as Cl_2) concentration in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).				
Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		Bromine (mg/L as Cl_2)		
C50-Control	T-0.0	0.11, 0.12	-0.36, -0.38	0.03, -0.10
	T-2.5	0.02, -0.02	0.03, -0.11	0.03, 0.08
	T-5.0	0.00, 0.00	-0.11, -0.09	0.02, 0.03
	T-7.5	Not Sampled	-0.01, -0.11	-0.14, -0.13
	T-10.0	Not Sampled	0.00, 0.00	-0.02, -0.06
D10-Control	T-0.0	0.01, 0.05	Not Sampled	Not Sampled
	T-2.5	0.22, 0.24	Not Sampled	Not Sampled
	T-5.0	-0.12, 0.00	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D30-Control	T-0.0	0.12, -0.02	Not Sampled	Not Sampled
	T-2.5	0.02, 0.02	Not Sampled	Not Sampled
	T-5.0	0.00, 0.16	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D50-Control	T-0.0	0.18, -0.02	Not Sampled	Not Sampled
	T-2.5	0.22, 0.00	Not Sampled	Not Sampled
	T-5.0	-0.44, 0.00	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
[1] Results for duplicate analyses				
[2] OR = Out of Range, > 5 mg/L as Cl_2				

The reaction of ozone with bromide ion results in the formation of hypobromous acid (HOBr, see Section 3.2 for more details on the chemistry). This is in equilibrium with hypobromite ion (OBr^-) with a pK_a of 8.8 (Haag and Hoigne, 1983). Therefore, the analysis of bromine, as reflected by the TRO measurement, provides a total concentration of bromine in mg/L of Br_2.

As was discussed in Section 3.2, ozone rapidly reacts with OBr^- to form Br^-; however, it does not react with HOBr. The pH of the ballast water in these experiments was variable but was approximately 7.5. Therefore, a substantial portion of the total bromine would be in the HOBr form and non-reactive with ozone. The results in Table 7.2.2 confirm this in that the concentration of bromine (or, more correctly, TRO) in all experiments increased with an increase in the time of ozonation. In experiments 2 and 3, where it is believed that more ozone was introduced into the ballast water as a result of better equipment operation, the concentration was over range (ca. 5 mg/L) in all samples after the T3, T4 and/or T5.

It is hard to speculate on the exact residual concentration on the ship; however, this high concentration may explain part of the observed effect on organisms where bromoform and toxicity increased in the WET tests.

7.3.2 Oxidation Reduction Potential.

One of the objectives of this study was to explore potential measurements that could eventually be used as monitoring and control functions. One such measurement was ORP. The data obtained for the three experiments are summarized in Table 7.3.2.1.

Table 7.3.2.1. Oxidation Reduction Potential (ORP), as measured using the laboratory electrode, of the samples in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).

Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		ORP (mV)		
A10- Treatment	T-0.0	129.5	77.1	71.6
	T-2.5	372.4	725.1	767.3
	T-5.0	718.9	774.3	761.6
	T-7.5	Not Sampled	781.7	782.1
	T-10.0	Not Sampled	789.5	794.9
A30- Treatment	T-0.0	140.2	69.4	75.6
	T-2.5	363.7	738.3	750.7
	T-5.0	738.6	782.6	785.1
	T-7.5	Not Sampled	793.2	791.7
	T-10.0	Not Sampled	796.4	788.2
A50- Treatment	T-0.0	136.8	72.5	95.7
	T-2.5	289.7	629.3	574.8
	T-5.0	753.0	792.0	713.9
	T-7.5	Not Sampled	787.4	785.5
	T-10.0	Not Sampled	797.5	793.2
B10- Treatment	T-0.0	115.7	74.3	89.3
	T-2.5	217.0	297.1	637.5
	T-5.0	385.7	748.2	754.2
	T-7.5	Not Sampled	774.7	781.4
	T-10.0	Not Sampled	784.7	793.2
B30- Treatment	T-0.0	144.6	77.0	92.6
	T-2.5	217.3	981.0	721.1
	T-5.0	506.6	765.6	774.6
	T-7.5	Not Sampled	776.2	786.3
	T-10.0	Not Sampled	785.5	798.7
B50- Treatment	T-0.0	162.2	75.7	95.8
	T-2.5	339.9	672.3	716.9
	T-5.0	495.6	762.6	772.9
	T-7.5	Not Sampled	779.0	790.9
	T-10.0	Not Sampled	793.9	799.0
C10-Control	T-0.0	201.3	97.3	108.0
	T-2.5	313.1	164.4	255.7
	T-5.0	379.1	244.4	293.9

Table 7.3.2.1. Oxidation Reduction Potential (ORP), as measured using the laboratory electrode, of the samples in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).

Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		ORP (mV)		
	T-7.5	Not Sampled	258.7	420.0
	T-10.0	Not Sampled	273.7	294.3
C30-Control	T-0.0	197.6	103.4	106.8
	T-2.5	312.1	193.4	256.8
	T-5.0	347.4	245.8	274.9
	T-7.5	Not Sampled	246.7	417.4
	T-10.0	Not Sampled	278.1	287.0
C50-Control	T-0.0	198.3	99.0	107.3
	T-2.5	292.2	183.7	253.9
	T-5.0	360.8	238.9	294.0
	T-7.5	Not Sampled	260.3	438.7
	T-10.0	Not Sampled	272.5	294.7
D10-Control	T-0.0	196.6	Not Sampled	Not Sampled
	T-2.5	308.9	Not Sampled	Not Sampled
	T-5.0	359.9	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D30-Control	T-0.0	198.6	Not Sampled	Not Sampled
	T-2.5	308.9	Not Sampled	Not Sampled
	T-5.0	359.9	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D50-Control	T-0.0	200.2	Not Sampled	Not Sampled
	T-2.5	306.0	Not Sampled	Not Sampled
	T-5.0	358.3	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled

[1] BMDL = Below Method Detection Limit

[2] Detection limit = 2 µg/L bromate ion

[3] Detection limit = 10 µg/L bromate ion

Comparing the total bromine concentrations and ORP measurements suggests that similarities exist in their concentrations as a function of ozonation (correlations not shown). Therefore, it is likely that the ORP measurement is recording the total oxidation state of the solution (but see also Section 7.10.1). In the case of the solutions that are ozonated, this is reflected in the measurement of total bromine. In the control ballast tanks, which also showed an increase in ORP with time, there are no similar correlations and the exact reason for this minor increase in ORP with sampling time are not clear. It may be that the probes used to measure ORP electrochemically "carry over" high mV signals for a short period of time even when placed into non-oxidized seawater.

7.3.3 Bromate Ion.

The bromate ion results are summarized in Table 7.3.3.1. The results indicate that bromate ion was always below the method detection limit in all samples. When bromate ion was spiked into the treated samples in the laboratory, the spike was never recovered fully. This result indicates that the water had a bromate ion demand. The cause of this apparent demand is not understood. However, it may be related to the high concentration of "active" bromine in the samples.

Table 7.3.3.1. Bromate ion data in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).

Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		Bromate Ion (µg/L)		
A10- Treatment	T-0.0	BMDL[1,2]	BMDL[3]	BMDL[3]
	T-2.5	BMDL	BMDL	BMDL
	T-5.0	BMDL	BMDL	BMDL
	T-7.5	Not Sampled	BMDL	BMDL
	T-10.0	Not Sampled	BMDL	BMDL
A30- Treatment	T-0.0	BMDL	BMDL	BMDL
	T-2.5	BMDL	BMDL	BMDL
	T-5.0	BMDL	BMDL	BMDL
	T-7.5	Not Sampled	BMDL	BMDL
	T-10.0	Not Sampled	BMDL	BMDL
A50- Treatment	T-0.0	BMDL	BMDL	BMDL
	T-2.5	BMDL	BMDL	BMDL
	T-5.0	BMDL	BMDL	BMDL
	T-7.5	Not Sampled	BMDL	BMDL
	T-10.0	Not Sampled	BMDL	BMDL
B10- Treatment	T-0.0	BMDL	BMDL	BMDL
	T-2.5	BMDL	BMDL	BMDL
	T-5.0	BMDL	BMDL	BMDL
	T-7.5	Not Sampled	BMDL	BMDL
	T-10.0	Not Sampled	BMDL	BMDL
B30- Treatment	T-0.0	BMDL	BMDL	BMDL
	T-2.5	BMDL	BMDL	BMDL
	T-5.0	BMDL	BMDL	BMDL
	T-7.5	Not Sampled	BMDL	BMDL
	T-10.0	Not Sampled	BMDL	BMDL
B50- Treatment	T-0.0	BMDL	BMDL	BMDL
	T-2.5	BMDL	BMDL	BMDL
	T-5.0	BMDL	BMDL	BMDL
	T-7.5	Not Sampled	BMDL	BMDL
	T-10.0	Not Sampled	BMDL	BMDL
C10-Control	T-0.0	BMDL	BMDL	BMDL

Table 7.3.3.1. Bromate ion data in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).				
Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		Bromate Ion (µg/L)		
	T-2.5	BMDL	BMDL	BMDL
	T-5.0	BMDL	BMDL	BMDL
	T-7.5	Not Sampled	BMDL	BMDL
	T-10.0	Not Sampled	BMDL	BMDL
C30-Control	T-0.0	BMDL	BMDL	BMDL
	T-2.5	BMDL	BMDL	BMDL
	T-5.0	BMDL	BMDL	BMDL
	T-7.5	Not Sampled	BMDL	BMDL
	T-10.0	Not Sampled	BMDL	BMDL
C50-Control	T-0.0	BMDL	BMDL	BMDL
	T-2.5	BMDL	BMDL	BMDL
	T-5.0	BMDL	BMDL	BMDL
	T-7.5	Not Sampled	BMDL	BMDL
	T-10.0	Not Sampled	BMDL	BMDL
D10-Control	T-0.0	BMDL	Not Sampled	Not Sampled
	T-2.5	BMDL	Not Sampled	Not Sampled
	T-5.0	BMDL	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D30-Control	T-0.0	BMDL	Not Sampled	Not Sampled
	T-2.5	BMDL	Not Sampled	Not Sampled
	T-5.0	BMDL	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D50-Control	T-0.0	BMDL	Not Sampled	Not Sampled
	T-2.5	BMDL	Not Sampled	Not Sampled
	T-5.0	BMDL	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
[1] BMDL = Below Method Detection Limit				
[2] Detection limit = 2 µg/L bromate ion				
[3] Detection limit = 10 µg/L bromate ion				

7.3.4 Bromoform.

The data for the concentration of bromoform in the three experiments are summarized in Table 7.3.4.1. In every experiment, the concentration of $CHBr_3$ increased with time. In those samples where a direct comparison could be made (that is from one experiment to another at the same time point), it is clear that the concentration of $CHBr_3$ increased more in Experiment 1 than in either 2 or 3. For all three experiments, the DOC was around 1 mg/L. Therefore, the differences were not due to a change in DOC concentration. In Experiment 3, the ozone-loading rate (i.e., the concentration or total ozone that was present) was higher than Experiment 1 or 2. The other variable that affects the amount of $CHBr_3$ formed is water temperature. It appears that this is the reason for the lower concentration of $CHBr_3$ in the two experiments that were conducted in November.

In general, three variables, the total amount of ozone delivered, DOC, and water temperature will affect the concentration of $CHBr_3$ that is formed. It is likely that ozonated water in the ballast tank upon standing (i.e., during the trip back to the port for a new cargo) will result in an increase in the concentration of $CHBr_3$. However, from the literature review, the concentration will not approach that, which would result in any toxicity to the receiving waters.

Table 7.3.4.1. Bromoform data in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).

Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		Bromoform (µg/L)		
A10- Treatment	T-0.0	BMDL [1,2]	BMDL [2]	BMDL [2]
	T-2.5	35.0	62.0	74.6
	T-5.0	136	77.4	77.7
	T-7.5	Not Sampled	91.2	93.0
	T-10.0	Not Sampled	92.2	90.1
A30- Treatment	T-0.0	BMDL	BMDL	BMDL
	T-2.5	30.0	68.4	80.0
	T-5.0	145	76.0	90.3
	T-7.5	Not Sampled	94.0	94.7
	T-10.0	Not Sampled	98.0	105.6
A50- Treatment	T-0.0	BMDL	BMDL	BMDL
	T-2.5	104	35.1	29.3
	T-5.0	Not Sampled	75.2	75.2
	T-7.5	Not Sampled	80.3	94.6
	T-10.0	Not Sampled	82.4	96.1
B10- Treatment	T-0.0	BMDL	BMDL	BMDL
	T-2.5	BMDL	32.9	42.5
	T-5.0	24.0	53.8	73.7
	T-7.5	Not Sampled	73.6	96.5
	T-10.0	Not Sampled	76.1	107
B30- Treatment	T-0.0	BMDL	BMDL	BMDL
	T-2.5	BMDL	44.6 (44.7) [3]	55.5
	T-5.0	47.2	70.4	70.6

Table 7.3.4.1. Bromoform data in the ballast tank treated with ozone (columns A and B) and the control tank (C and D).

Sample Location	Sample Time	Experiment 1	Experiment 2	Experiment 3
		Bromoform (µg/L)		
	T-7.5	Not Sampled	75.7	96.5
	T-10.0	Not Sampled	83.0	103
B50- Treatment	T-0.0	BMDL	BMDL	BMDL
	T-2.5	BMDL	40.4	46.2
	T-5.0	35.8	58.7	87.1
	T-7.5	Not Sampled	74.8	79.0
	T-10.0	Not Sampled	79.4	105
C10-Control	T-0.0	BMDL	BMDL	BMDL
	T-2.5	BMDL	BMDL	BMDL
	T-5.0	BMDL	BMDL	BMDL
	T-7.5	Not Sampled	BMDL(BMDL)	BMDL
	T-10.0	Not Sampled	BMDL	BMDL
C30-Control	T-0.0	BMDL	BMDL	BMDL
	T-2.5	Not Sampled	BMDL	BMDL
	T-5.0	BMDL	BMDL	BMDL
	T-7.5	Not Sampled	BMDL(BMDL)	BMDL
	T-10.0	Not Sampled	BMDL	BMDL
C50-Control	T-0.0	BMDL	BMDL	BMDL
	T-2.5	BMDL	BMDL	BMDL
	T-5.0	BMDL	BMDL	BMDL
	T-7.5	Not Sampled	BMDL	BMDL
	T-10.0	Not Sampled	BMDL	BMDL
D10-Control	T-0.0	BMDL	Not Sampled	Not Sampled
	T-2.5	BMDL	Not Sampled	Not Sampled
	T-5.0	BMDL	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D30-Control	T-0.0	BMDL	Not Sampled	Not Sampled
	T-2.5	BMDL	Not Sampled	Not Sampled
	T-5.0	BMDL	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D50-Control	T-0.0	BMDL	Not Sampled	Not Sampled
	T-2.5	BMDL	Not Sampled	Not Sampled
	T-5.0	BMDL	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled

[1] BMDL = Below Method Detection Limit

[2] Detection limit = 5 µg/L bromoform

7.4 Bacteria

7.4.1 Culturable Heterotrophic Plate Counts of Treated and Control Ballast Water

Results of the heterotrophic plate count from the Phase 1 study are displayed in Tables 7.4.1.1 and 7.4.1.2. The counts displayed are from either the direct spread plate method or the membrane filtration method for each sample. The numbers presented are selected from the method that provided the best range of countable colonies for the sample. For example, for the ozonated seawater samples, the ozone treatment method was very effective in inactivating culturable heterotrophic bacteria. If 100 µl aliquots of treated seawater were inoculated onto the surface of Marine R2A Agar by the spread plate method, there would typically be no colonies that would grow. Therefore, the culturable microorganisms were concentrated by using a membrane filtration method so the sensitivity of the enumeration assay could be increased. The numbers shown in Tables 7.4.1.1 and 7.4.1.2 are an average of the plating performed in triplicate or duplicate for each diluted or original portion of the seawater sample.

Table 7.4.1.1 displays the number of colony forming units that were found in treated and untreated water samples collected from *S/T Tonsina* during September and November 2001. The number of culturable microorganisms was between 10^5 and 10^6 colony forming units per liter before the ballast water was ozonated and throughout the duration of the experiment in the control ballast tank. After ozonation, the number of viable organisms declined by the first 2.5 hour sample. The decline was much greater in Experiments 2 and 3, compared to Experiment 1. In Experiments 2 and 3, the number of bacteria declined to 10^2 and 10^3 colony forming units per liter in samples collected from column B and the 50 foot sample from column A in the ozonated ballast tank. Bacteria in the 10 and 30-foot sample in column A declined to 10^0 to 10^1 colony-forming units per liter.

Following 5.0 hours of treatment, the bacteria populations continued to decrease in experiments 2 and 3 to a number below the detection limit (5 colony-forming units per liter) and 40 colony-forming units per liter. One-third of the samples collected after 5.0 hours contained bacterial levels less than the level of detection. Samples collected at 7.5 and 10 hours contained very few viable cells, if any viable cells were found at all.

7.4.2 Culturable Heterotrophic Plate Counts for Treated and Untreated Ballast Water Stored for up to 35 Days

A small experiment was conducted with treated and untreated ballast water that was collected during Experiment 1 and Experiment 3. This seawater was placed in 10-L sterile carboys and returned to the University of Washington for storage at 10°C. Aliquots of water were removed from the carboys following 2, 7, and 35 days. In Experiment 1, the number of culturable bacteria was below the level of detection when the treated sample was analyzed following 2 days of storage. In Experiment 3, the length of storage was extended to 35 days. In this experiment, bacterial numbers in the control sample remained elevated throughout the entire period of storage, between 10^5 and 10^6 colony forming units. For the treated ballast water, the number of culturable bacteria remained below the level of detection, 3 colony forming units per liter, for samples analyzed following 2, 7, and 35 days of storage.

Table 7.4.1.1. Enumerations of culturable heterotrophic bacteria from treated and control *S/T Tonsina* ballast tanks.				
		Colony forming units (CFU) / L		
Sample Location	Time	Experiment 1 9/01	Experiment 2 11/01	Experiment 3 11/01
A10- Treatment	T-0.0	4.70×10^{6}	1.30×10^{6}	4.10×10^{5}
	T-2.5	1.00×10^{4}	1.00×10^{1}	1.00×10^{1}
	T-5.0	$<3.00 \times 10^{3}$	4.00×10^{1}	5.00×10^{0} *
	T-7.5	Not Sampled	$<3.00 \times 10^{0}$	5.00×10^{0} *
	T-10.0	Not Sampled	$<3.00 \times 10^{0}$	$<5.00 \times 10^{0}$ *
A30- Treatment	T-0.0	2.70×10^{6}	9.20×10^{5}	2.40×10^{5}
	T-2.5	3.00×10^{3}	3.00×10^{1}	7.00×10^{0}
	T-5.0	$<3.00 \times 10^{3}$	3.00×10^{0}	$<5.00 \times 10^{0}$ *
	T-7.5	Not Sampled	3.00×10^{0}	$<5.00 \times 10^{0}$ *
	T-10.0	Not Sampled	$<3.00 \times 10^{0}$	5.00×10^{0} *
A50- Treatment	T-0.0	2.30×10^{6}	9.30×10^{5}	3.20×10^{5}
	T-2.5	$<3.00 \times 10^{3}$	5.80×10^{2}	6.00×10^{2}
	T-5.0	$<3.00 \times 10^{3}$	$<3.00 \times 10^{0}$	2.00×10^{1} *
	T-7.5	Not Sampled	1.00×10^{1}	$<5.00 \times 10^{0}$ *
	T-10.0	Not Sampled	$<3.00 \times 10^{0}$	5.00×10^{0} *
B10- Treatment	T-0.0	1.64×10^{7}	9.40×10^{5}	3.60×10^{5}
	T-2.5	1.09×10^{6}	9.00×10^{2}	1.20×10^{3}
	T-5.0	3.00×10^{3}	4.00×10^{1}	5.00×10^{0} *
	T-7.5	Not Sampled	1.00×10^{1}	$<5.00 \times 10^{0}$ *
	T-10.0	Not Sampled	1.00×10^{1}	$<5.00 \times 10^{0}$ *
B30- Treatment	T-0.0	3.20×10^{6}	8.70×10^{5}	3.20×10^{5}
	T-2.5	6.40×10^{5}	5.00×10^{2}	1.30×10^{3}
	T-5.0	$<3.00 \times 10^{3}$	3.00×10^{1}	7.00×10^{0}
	T-7.5	Not Sampled	$<3.00 \times 10^{0}$	5.00×10^{0} *
	T-10.0	Not Sampled	$<3.00 \times 10^{0}$	$<5.00 \times 10^{0}$ *
B50- Treatment	T-0.0	1.10×10^{6}	8.50×10^{5}	5.20×10^{5}
	T-2.5	2.40×10^{5}	3.00×10^{2}	1.10×10^{3}
	T-5.0	3.00×10^{3}	4.00×10^{1}	7.00×10^{0}
	T-7.5	Not Sampled	1.00×10^{1}	5.00×10^{0} *
	T-10.0	Not Sampled	$<3.00 \times 10^{0}$	$<5.00 \times 10^{0}$ *
C10-Control	T-0.0	2.30×10^{6}	1.10×10^{6}	7.00×10^{5}

Table 7.4.1.1. Enumerations of culturable heterotrophic bacteria from treated and control *S/T Tonsina* ballast tanks.

		Colony forming units (CFU) / L		
Sample Location	Time	Experiment 1 9/01	Experiment 2 11/01	Experiment 3 11/01
	T-2.5	1.10×10^6	3.70×10^7	6.40×10^5
	T-5.0	6.00×10^5	8.40×10^5	7.20×10^5
	T-7.5	Not Sampled	7.90×10^5	6.70×10^5
	T-10.0	Not Sampled	7.60×10^5	6.20×10^5
C30-Control	T-0.0	1.70×10^6	7.70×10^5	2.30×10^5
	T-2.5	9.00×10^5	3.30×10^7	6.60×10^5
	T-5.0	8.00×10^5	7.90×10^5	5.70×10^5
	T-7.5	Not Sampled	7.70×10^5	6.00×10^5
	T-10.0	Not Sampled	7.40×10^5	6.30×10^5
C50-Control	T-0.0	9.00×10^5	7.60×10^5	3.20×10^5
	T-2.5	7.00×10^5	8.70×10^5	7.40×10^5
	T-5.0	5.00×10^5	8.90×10^5	6.60×10^5
	T-7.5	Not Sampled	7.80×10^5	6.70×10^5
	T-10.0	Not Sampled	8.80×10^5	7.60×10^5
D10-Control	T-0.0	9.00×10^5	Not Sampled	Not Sampled
	T-2.5	7.00×10^5	Not Sampled	Not Sampled
	T-5.0	8.00×10^5	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D30-Control	T-0.0	8.00×10^5	Not Sampled	Not Sampled
	T-2.5	5.00×10^5	Not Sampled	Not Sampled
	T-5.0	6.00×10^5	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled
D50-Control	T-0.0	5.00×10^5	Not Sampled	Not Sampled
	T-2.5	5.00×10^5	Not Sampled	Not Sampled
	T-5.0	4.00×10^5	Not Sampled	Not Sampled
	T-7.5	Not Sampled	Not Sampled	Not Sampled
	T-10.0	Not Sampled	Not Sampled	Not Sampled

* = Sample enumerated in duplicate instead of triplicate

- = Sample not analyzed.

Table 7.4.1.2. Enumeration of culturable heterotrophic bacteria in treated and untreated *S/T Tonsina* ballast water following 2, 7 and 35 days of storage of the seawater after the end of the ship-board ozone treatment experiment.				
2 days of Storage		Colony forming units (CFU) / L		
Sample Location	Time	Experiment 1	Experiment 2	Experiment 3
Control	T3	1.20×10^{6}	Not Sampled	-
Treatment		$<3.00 \times 10^{3}$	Not Sampled	$<3.00 \times 10^{0}$
Control	T5	Not Sampled	Not Sampled	6.00×10^{5}
Treatment		Not Sampled	Not Sampled	$<3.00 \times 10^{0}$
7 days of Storage				
Sample Location	Time	Experiment 1	Experiment 2	Experiment 3
Control	T3	Not Sampled	Not Sampled	-
Treatment		Not Sampled	Not Sampled	$<3.00 \times 10^{0}$
Control	T5	Not Sampled	Not Sampled	4.60×10^{6}
Treatment		Not Sampled	Not Sampled	$<3.00 \times 10^{0}$
35 days of Storage				
Sample Location	Time	Experiment 1	Experiment 2	Experiment 3
Control	T3	Not Sampled	Not Sampled	-
Treatment		Not Sampled	Not Sampled	$<3.00 \times 10^{0}$
Control	T5	Not Sampled	Not Sampled	2.00×10^{6}
Treatment		Not Sampled	Not Sampled	$<3.00 \times 10^{0}$
- = Sample not analyzed.				

7.5 Zooplankton

In the 5-hour ozone exposure experiment conducted on 24 September, the average percent of animals alive was uniformly high (range: 94-97%) in pre-treatment samples (Table 7.5.1). Mortality after five hours was different for the two treatment columns: column A had 91% mortality, and column B showed 47% mortality.

While the September zooplankton assemblage was dominated by the calanoid copepod *Paracalanus* sp., it was quite diverse, with several other relatively numerous copepod taxa as well as numerous planktonic larvae of barnacles, polychaetes, and other animals. After 5 hours of ozone exposure, the poecilostomatoid copepod *Corycaeus anglicus* and large Cirripedia (barnacle) nauplii appeared to be particularly unaffected by the treatment. On the other hand, small calanoid copepod nauplii larvae appeared to experience relatively higher mortality than did other organisms.

Table 7.5.1. Results of September 24 zooplankton ozone mortality experiment (n = 3).

9/24/2001	Average Percent Alive	SD	Average Percent Moribund	SD	Average Percent Dead	SD
			Pre-treatment			
Column A-treatment	93.7	0.6	5.7	1.5	0.7	1.2
Column B-treatment	95.3	1.2	3.7	2.1	1.0	1.0
Column C-control	97.0	2.0	1.7	0.6	1.3	1.5
Column D-control	95.7	1.5	3.0	1.0	0.3	0.6
			5 hours treatment			
Column A-treatment	1.7	0.6	7.3	3.1	91.0	3.0
Column B-treatment	25.0	4.0	27.7	0.6	47.3	3.5
Column C-control	92.3	1.5	5.3	2.3	2.3	1.5
Column D-control	92.7	2.9	6.0	2.6	1.3	0.6

The November 2 10-hours experiment had results similar to the September experiment, in showing differential mortality at 5 hours between the two treated columns (Table 7.5.2). In contrast to the September experiment, survival was higher in column A than in column B. Mortality after 5 hours was also lower in this experiment than in the September experiment (20 % vs. 47 % in the "high survival" column, 66 % vs. 91 % in the "low survival" column). After 10 hours of treatment, the pattern of differential mortality between the treatment columns persisted, although mortality increased.

Table 7.5.2. Results of November 2 zooplankton ozone mortality experiment (n = 3).

11/02/2001	Average Percent Alive	SD	Average Percent Moribund	SD	Average Percent Dead	SD
			Pre-treatment			
Column A-treatment	96.3	1.2	3.0	0.0	0.7	1.2
Column B-treatment	93.7	1.5	4.0	1.7	0.3	0.6
Column C-control	97.3	2.1	1.3	1.2	1.7	2.1
			5-hours treatment			
Column A-treatment	40.3	3.2	39.7	8.5	20.0	6.2
Column B-treatment	13.7	2.5	20.0	6.0	66.3	8.5
Column C-control	97.7	1.5	2.3	1.5	0.0	0.0
			10-hours treatment			
Column A-treatment	13.7	1.5	19.3	8.7	67.0	9.6
Column B-treatment	1.7	1.2	1.0	1.0	97.3	2.1
Column C-control	94.3	3.8	5.0	3.6	0.7	0.6

In the November 4 experiment, differences in mortality between the two treatment columns was far less marked, and mortality appeared much higher at both treatment times than in the other experiments (Table 7.5.3).

In the November experiments, taxonomic diversity was much lower than in September, and the zooplankton assemblage was largely dominated by late juvenile stages of the calanoid copepod *Paracalanus* sp. Each plankton tow contained several specimens of the Asian calanoid copepod *Pseudodiaptomus marinus*. As none of these species were found in plankton tows from Port Angeles harbor taken both day and night, during the November 2 experiment, it was presumed that the individual organisms observed represented remnants of ballast water from the ship's last voyage to Long Beach harbor, where *P. marinus* has been introduced. This species, the harpacticoid copepod *Microsetella* sp., and nematode worms appeared to be relatively resistant to ozone treatment as compared to the *Paracalanus* sp.

Table 7.5.3. Results of November 4 zooplankton ozone mortality experiment (n = 3).

11/04/2001	Average Percent Alive	SD	Average Percent Moribund	SD	Average Percent Dead	SD
			Pre-treatment			
Column A-treatment	89.7	7.0	6.0	2.6	7.7	6.8
Column B treatment	94.7	2.5	2.3	1.5	3.0	1.0
Column C control	93.3	4.0	3.7	0.6	3.0	3.6
			5 hours treatment			
Column A-treatment	7.7	5.7	8.3	4.2	84.0	7.0
Column B-treatment	1.7	1.2	6.0	2.0	92.3	3.1
Column C-control	97.0	1.0	1.0	1.0	3.3	1.2
			10 hours treatment			
Column A-treatment	1.3	2.3	2.0	2.0	96.7	3.1
Column B-treatment	0.0	0.0	0.7	1.2	99.3	1.2
Column C-control	93.3	1.5	2.3	0.6	4.3	1.5

7.6 Phytoplankton

During both experiments, dinoflagellate populations exhibited sharp decreases in the ozone treatment tank relative to the control tank (Figures 7.6.1 and 7.6.2). For the 2 November experiment, samples collected 10 hours after ozone treatment contained 0-18% of the initial concentrations of dinoflagellates at column A (with concentration increasing with depth) and 0% of initial concentrations of dinoflagellates at column B. For the 4 November experiment, dinoflagellates were not detected at all in the ozone treatment tank, creating estimates of 0% of the initial concentrations remaining 10 hours post-treatment for all depths. In contrast, dinoflagellate concentrations did not exhibit any clear decline in the control tank in either experiment, ranging from 70 - 745% of the initial concentration after 10 hours.

Microflagellate concentrations exhibited a similar pattern between treatments (Tables 7.6.1 and 7.6.2). Ten hours after treatment, microflagellate concentrations declined between 1-30% in column A and between 2-7% in column B during the 2 November experiment. Interestingly, the smaller decline in column A was also observed for dinoflagellates during this experiment, suggesting a spatial variation in performance of ozone treatment within this experimental run. In contrast, no such spatial variation was evident for the second experiment on

4 November, and microflagellates declined to 1-4% of initial concentrations. For both dates, no appreciable decline was evident in the control tank for microflagellates.

In general, the results suggest ozone treatment has a very strong effect on vegetative cells of dinoflagellates and microflagellates. The observed decline is probably due to mortality, whereby the vegetative cells are simply destroyed by ozonation. Some portion of this decline could result from sedimentation, but we did not measure the possible accumulation of cells or resting stages at the bottom during this first phase of work. However, because sedimentation would also have occurred in the control tank, mortality from ozone exposure is still the most likely explanation for reduced populations densities of dinoflagellates and microflagellates in the treatment tank.

The results for diatoms are much more difficult to interpret. For the 2 November experiment, diatom concentrations varied from 17-135% of the initial concentrations after 10 hours in the ozone treatment tank. For the 4 November experiment, similar measures ranged from 20-120%. On both dates, no clear decline in abundance was observed in the control tank over the same time course.

Although the results for diatoms suggest that ozone treatment may be much less effective on these organisms compared to the other two groups, this likely represents a limitation of microscopic methods used during this phase of analyses. More specifically, unlike the dinoflagellates and microflagellates, diatoms are identified on the basis of the shape and patterns of their silica cell walls (frustules) that will not decompose quickly when exposed to ozone. Thus, although present in direct counts in relatively high numbers (following treatment), it is not possible to determine whether the diatoms counted were dead or alive with the method used.

Overall, the phytoplankton results show considerable promise for ozone treatment to remove phytoplankton from ballast water. Clearly more replication is required, under a range of conditions, to test how successful this approach may be. Furthermore, additional measures are needed in the next phase to test for possible accumulation of phytoplankton in bottom sediments and distinguish live from dead diatoms.

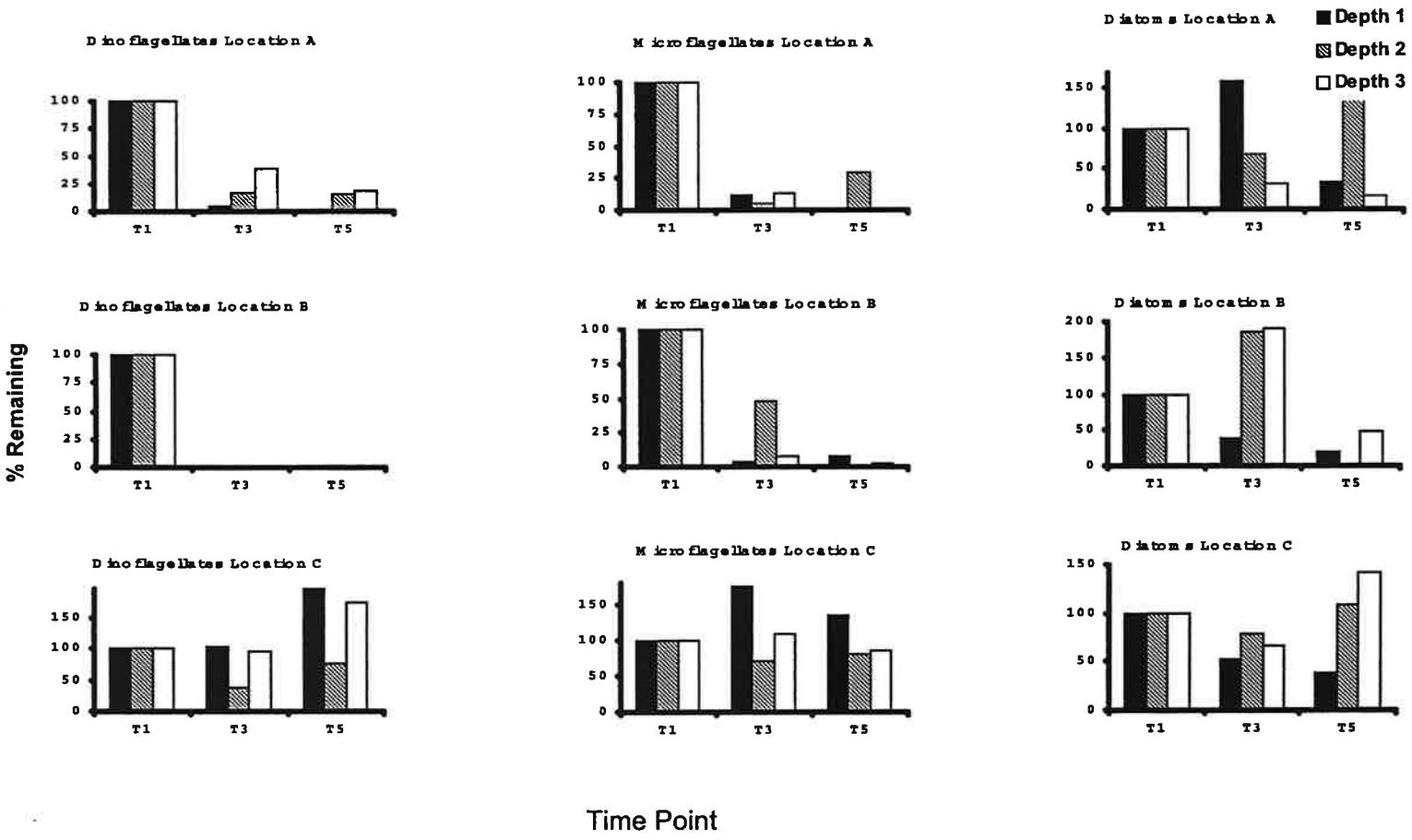

Figure 7.6.1. Change in concentrations of dinoflagellates, microflagellates, and diatoms during the ozone treatment experiment on 2 November 2001. Shown for each of 3 tank locations is the percentage of initial concentrations present at 3 depths. Percent change is relative to that measured at Time Point T1 for each tank and depth. Locations (i.e., columns) A and B were within the same tank that received ozone treatment, whereas location C was in a separate (control) tank, which did not receive ozone treatment. Samples at Time Point T1 were collected prior to ozone treatment, and Time Points T3 and T5 were collected at 5 and 10 hours, respectively, after ozone treatment was initiated. [Note: Sample lost for Location B, Time Point 5, Depth 2.]

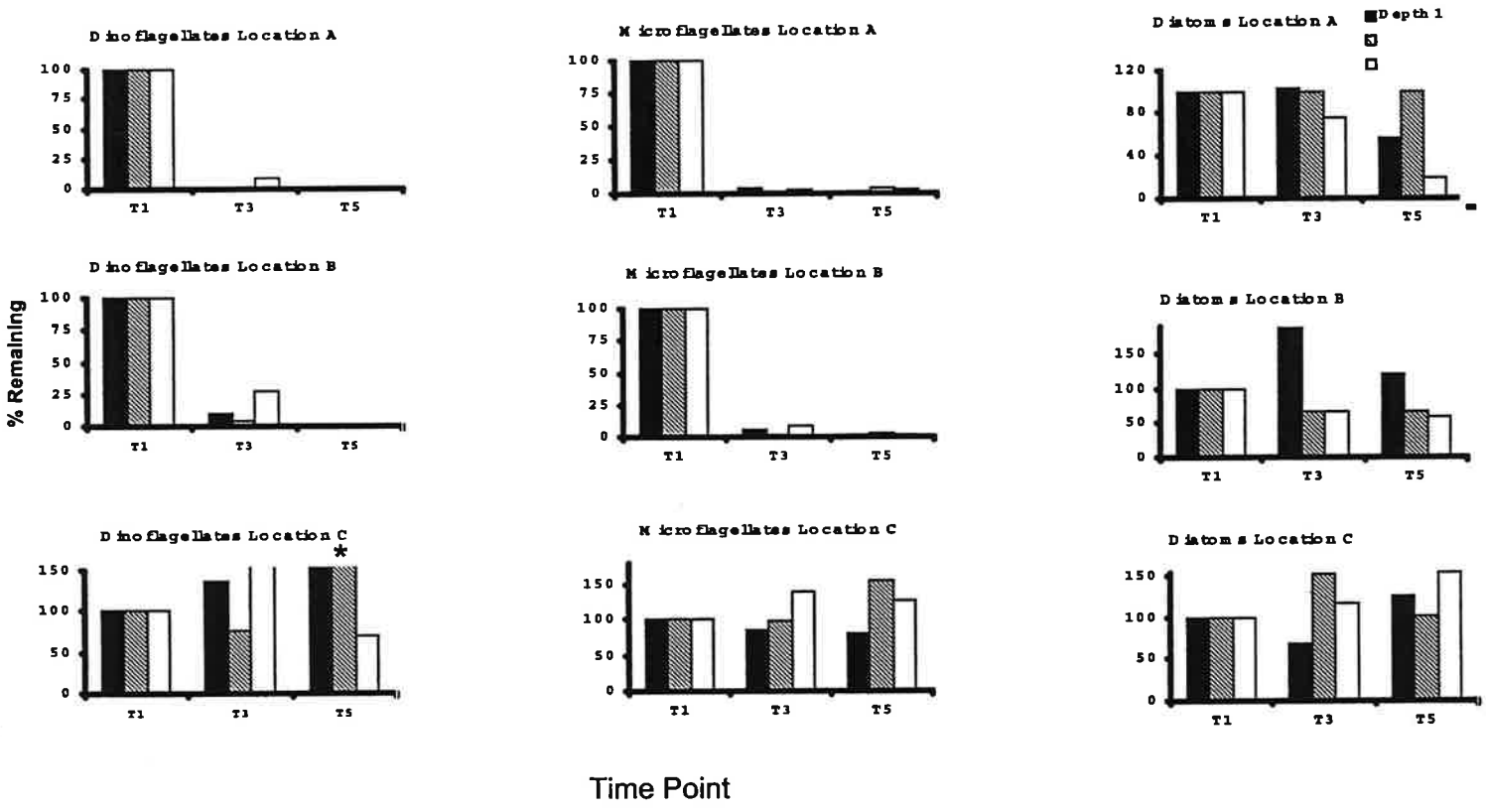

Figure 7.6.2. Change in concentrations of dinoflagellates, microflagellates, and diatoms during the ozone treatment experiment on 4 November 2001. Shown for each of 3 tank locations is the percentage of initial concentrations present at 3 depths. Percent change is relative to that measured at Time Point T1 for each tank and depth. Locations A and B were within the same tank that received ozone treatment, whereas location C was in a separate (control) tank, which did not receive ozone treatment. Samples at Time Point T1 were collected prior to ozone treatment, and Time Points T3 and T5 were collected at 5 and 10 hours, respectively, after ozone treatment was initiated. Asterisk indicates values that exceed the y-axis.

7.7 Caged Organism Experiments

7.7.1 Experiment 1

The first of three experiments exposed caged organisms in two ozone-treated columns and in two control columns for a 5-hour duration. Survival of control organisms was essentially 100 % (only 1 of 30 amphipods died, but 3 exposure chambers in Column C were lost) (Table 7.7.1.1). Survival was also 100% in ozone treated ballast tank Column B (aft column) for all species. In ozone Column A (fore column), mysid survival ranged from 20-60 % and sheepshead survival ranged from 0-30 %. Survival for both of these species was directly related to depth; those closest to the bottom suffered the highest mortality (Fig 7.7.1.1). Most of the surviving sheepshead and mysids were moribund (Table 7.7.1.1).

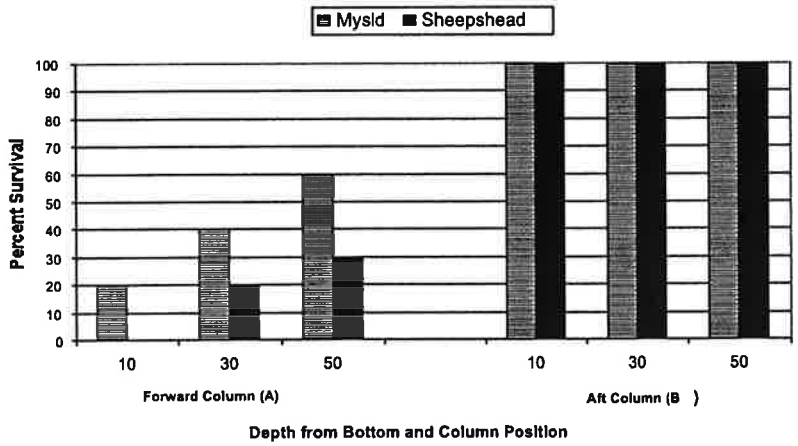

Figure 7.7.1.1. Survival of caged organisms in the forward ozone exposure column (A) during the first experiment (5-hour exposure).

Results of this first experiment suggested that: 1) the selected species showed a range in sensitivity to ozone, 2) toxicity was apparent in the forward ozone column (A), but absent in the aft column (B), and 3) toxicity may vary with depth in the water column. These observations suggested that a longer ozonation exposure time is necessary (or a higher ozone concentration is needed for the same exposure time) to affect high mortality for these species, and that the ozone system did not provide uniform ozone distribution laterally or vertically within the ballast tanks.

Table 7.7.1.1. Experiment 1 caged organism percent survival and percent moribund after 5 hours of ozone or control exposure.

		Percent Survival at 5 Hours				Percent Moribund at 5 Hours			
Treatment/Column	Depth*	Mysid	Sheepshead	Shore Crab	Amphipod	Mysid	Sheepshead	Shore Crab	Amphipod
Ozone A (fore)	0				100				0
"	0				100				0
"	0				100				0
"	10	20	0	100		0	All dead	0	
"	30	40	20	100		100	100	0	
"	50	60	30	100		83.3	33.3	0	
Ozone B (aft)	0				100				0
"	0				100				0
"	0				100				0
"	10	100	100	100		0	0	0	
"	30	100	100	100		0	0	0	
"	50	100	100	100		0	0	0	
Control (fore)	0				Chambers lost				Chambers lost
"	0				Chambers lost				Chambers lost
"	0				Chambers lost				Chambers lost
"	10	100	100	100		0	0	0	
"	30	100	100	100		0	0	0	
"	50	100	100	100		0	0	0	
Control D (aft)	0				100				0
"	0				90				0
"	0				100				0
"	10	100	100	100		0	0	0	
"	30	100	100	100		0	0	0	
"	50	100	100	100		0	0	0	

* Depth = distance in feet from the bottom of the tank

7.7.2 Experiment 2

The second experiment exposed all test organisms to a 10-hour ozonation duration in three treatment columns (fore, middle and aft) and to control conditions in only one column. For this experiment, control survival was essentially 100% for all species (only 1 of 30 mysids died), and none showed signs of any adverse effects (Table 7.7.2.1). For animals exposed to ozone in the three treatment columns, average percent survival by species was: mysid 51.4%, sheepshead 12.2%, shore crab 100.0%, and amphipod 92.2%. Once again, survival was a function of depth in the water column. Survival was the highest at the 10-foot station (closest station to the bottom), and decreased higher in the water column (30 and 50 foot stations; Figure 7.7.2.1; Table 7.7.2.1). This survival pattern, relative to depth, is opposite of that seen in Experiment 1. Degree of survival was also a function of column location: survival was lowest (and essentially equal) in the fore and middle columns, and was greatest in the aft column (Figure 7.7.2.1). In Experiment 2, all shore crabs survived and amphipods suffered only slight mortality (Figure 7.7.2.1). Of the surviving animals, the average percentage moribund was: sheepshead 88%, mysid 10%, amphipod 16%, and shore crab 0% (Table 7.7.2.1).

Results of the second experiment helped to clarify the relative sensitivity of the four test species. Their relative rank in sensitivity to ozone is: sheepshead > mysids > amphipods > shore crabs (Figure 7.7.2.1). The lateral pattern of toxicity was the same for Experiments 1 and 2: toxicity was highest in the fore columns and least in the aft columns. However, the vertical pattern of toxicity was different between Experiments 1 and 2; toxicity was lowest near the bottom in Experiment 2, which was the opposite of Experiment 1. This difference in vertical toxicity between the first two experiments may be due to a change in the ozone concentration in the water column.

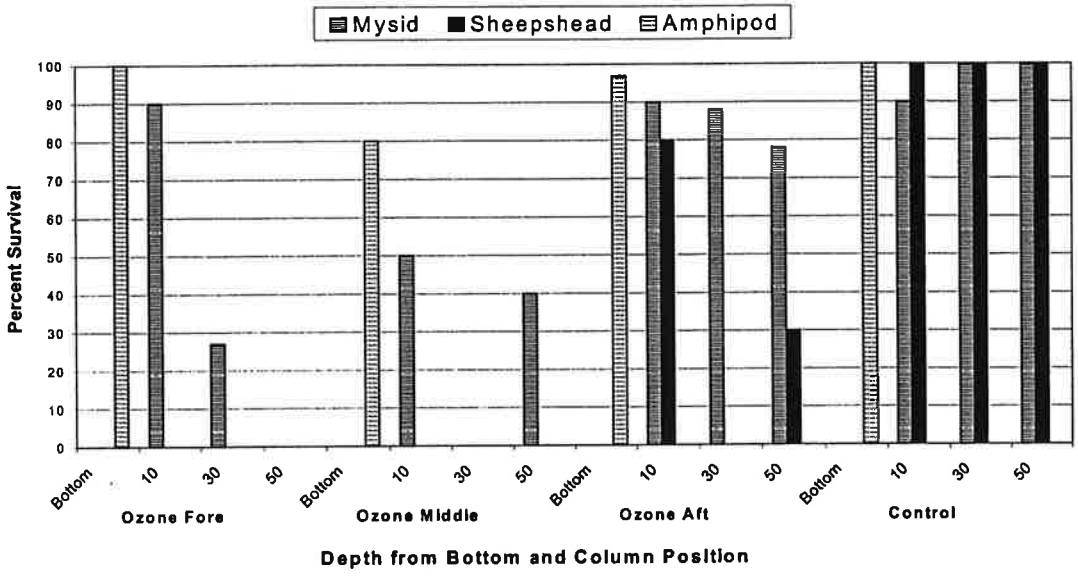

Figure 7.7.2.1. Average percent survival of caged organisms by depth and column position during the second experiment (10-hour exposure).

Table 7.7.2.1. Experiment 2 caged organism percent survival and percent moribund after 10 hours of ozone or control exposure.

		Percent Survival at 10 Hours				Percent Moribund at 10 Hours		
Treatment/Column	Depth*	Mysid	Sheepshead	Shore Crab	Amphipod	Mysid	Sheepshead	Shore Crab
Ozone 1 (fore)	0				100			
"	0				100			
"	0				100			
"	10	90	0	100		0	All dead	0
"	30	27	0	100		0	All dead	0
"	50	0	0	100		All dead	All dead	0
Ozone 4 (middle)	0				100			
"	0				90			
"	0				50			
"	10	50	0	100		0	All dead	0
"	30	0	0	100		All dead	All dead	0
"	50	40	0	10		0	All dead	0
Ozone 7 (aft)	0				100			
"	0				100			
"	0				90			
"	10	90	80	100		11.1	75	0
"	30	88	0	100		28.6	All dead	0
"	50	78	30	100		28.6	100	0
Control 3	0				100			
"	0				100			
"	0				100			
"	10	90	100	100		0	0	0
"	30	100	100	100		0	0	0
"	50	100	100	100		0	0	0

* Depth = distance in feet from the bottom of the tank

7.7.3 Experiment 3

The third experiment again exposed all test organisms for 10 hours in the fore, middle, and aft columns and to control conditions in one column. Control survival for this experiment was 100% and none of the control animals showed signs of stress (Table 7.7.3.1). For animals exposed to ozone, average percent survival by species was: mysid 31.1, sheepshead 0.0, shore crab 100.0 and amphipod 93.3. Many of the surviving mysids and about 15 % of the surviving amphipods appeared moribund (Table 7.7.3.1). Also, in the ozone exposure, all of the surviving shore crabs appeared to be moving sluggishly, a moribund state. For Experiment 3, there was no obvious trend in survival rates as a function of depth (Figure 7.7.3.1), although only mysids suffered partial kills, so data with which to make either vertical or lateral comparisons were sparse. As for the previous two experiments, survival (for mysids) was highest in the aft column. Once again, amphipods contained in the bottom buckets suffered only slight mortality (Table 7.7.3.1).

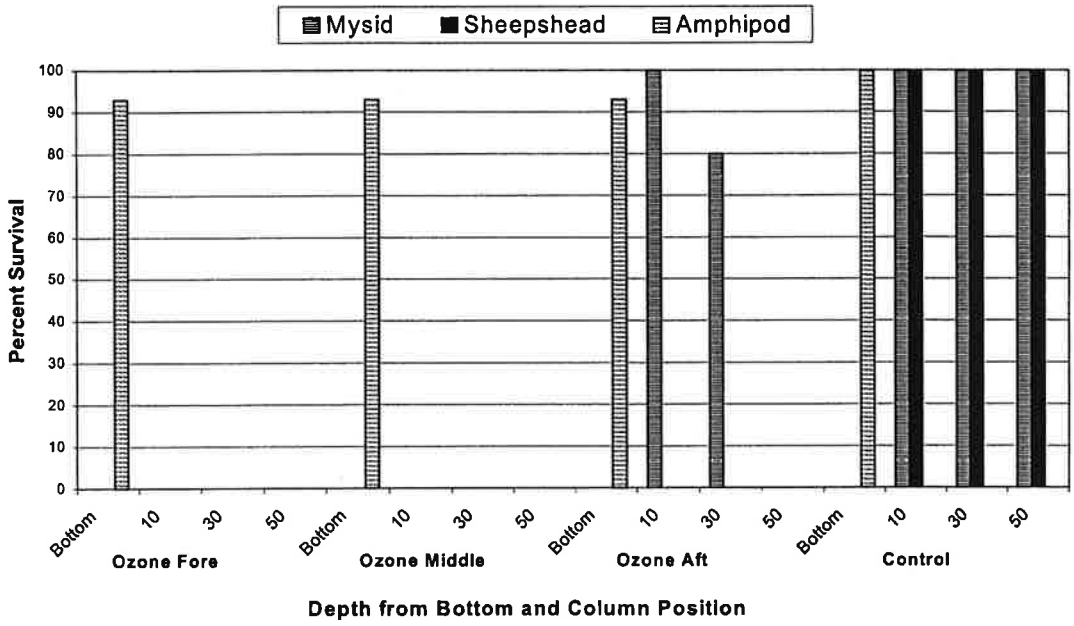

Figure 7.7.3.1. Average percent survival of caged organisms by depth and column position during the second experiment (10-hour exposure).

Table 7.7.3.1. Experiment 3 caged organism percent survival and percent moribund after 10 hours of ozone or control exposure.

		Percent Survival at 10 Hours				Percent Moribund at 10 Hours		
Treatment/Column	Depth*	Mysid	Sheepshead	Shore Crab	Amphipod	Mysid	Sheepshead	Shore Crab
Ozone 1 (fore)	0				100			
"	0				100			
"	0				80			
"	10	0	0	100		0	All dead	100
"	30	0	0	100		0	All dead	100
"	50	100	0	100		0	All dead	100
Ozone 4 (middle)	0				100			
"	0				100			
"	0				80			
"	10	0	0	100		0	All dead	100
"	30	0	0	100		0	All dead	100
"	50	0	0	100		0	All dead	100
Ozone 7 (aft)	0				100			
"	0				100			
"	0				80			
"	10	100	0	100		0	All dead	100
"	30	80	0	100		100	All dead	100
"	50	0	0	100		0	All dead	100
Control 3	0				100			
"	0				100			
"	0				100			
"	10	100	100	100		0	0	0
"	30	100	100	100		0	0	0
"	50	100	100	100		0	0	0

* Depth = distance in feet from the bottom of the tank

Starting with the most sensitive species, the results of Experiment 3 once again confirmed that the order of relative species sensitivity to ozone is sheepshead, mysids, amphipods, and finally shore crabs. Toxicity of ozone to the sheepshead and mysids was highest in Experiment 3. Also, differences in toxicity as a function of both depth and column position appeared to be less in this experiment, suggesting that increased ozone resulted in a more even distribution of the ozone and active bromine throughout the water column. Indeed, ozone loading rates in the vertical portion of the ozonated ballast tank (where the caged organisms were exposed) rose from 0.59 mg ozone/L/hour in Experiment 1 to 0.86 and 1.35 mg ozone/L/hour in Experiments 2 and 3, respectively.

7.7.4 Correlations

Mysids, being of intermediate sensitivity, gave the best "partial kill" data with which to calculate correlation coefficients with water chemistry parameters measured during the test. Mortality data were only collected at the end of each test (at 5 hours for Experiment 1, 10 hours for Experiments 2 and 3), while "ozone measurements" (i.e., ozone, bromine, ORP) were collected at 2.5-hour intervals.

The highest correlation was between mysid mortality and ORP measured next to the cages with the Hydrolab probe (all other measurements were collected in columns adjacent to the caged organisms; Table 7.7.4.1). The next highest correlation with mysid mortality was generally with bromine concentration, followed by ORP measured with the laboratory probe. Ozone concentrations showed very little correlation with mysid mortality (Table 7.7.4.1).

Table 7.7.4.1. Correlation coefficients (r) between end-of-test mysid mortality and ozone, bromine and ORP measurements at 2.5-hour intervals.

	Sampling Hour			
Parameter	2.5	5	7.5	10
Ozone	-0.0750	-0.3282	-0.1889	0.1284
Bromine	0.6689	0.4049	0.6302	OR*
Lab Probe ORP	0.3756	0.4558	0.1805	-0.1752
Hydrolab ORP	0.7287	0.7429	0.6329	0.8178

*All measurements were "over range"